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Effects of Fish Introductions on the Geographic Distribution and Native Invertebrate Biodiversity of Naturally Fishless Lakes in Maine

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EFFECTS OF FISH INTRODUCTIONS ON THE GEOGRAPHIC DISTRIBUTION AND NATIVE INVERTEBRATE BIODIVERSITY OF NATURALLY FISHLESS LAKES IN MAINE

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By Emily Gaenzle Schilling

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An Abstract of the Thesis Presented
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Widespread fish stocking has led to a worldwide decline in naturally fishless lakes and their associated communities. Little is known about the historical distribution or native communities of these freshwater ecosystems. The objectives of this study were to: 1) develop a quantitative method to remotely detect naturally fishless lakes in Maine, 2) conduct a landscape-scale assessment of unique attributes of fishless lake macroinvertebrate communities, 3) identify macroinvertebrate bioindicators of fish absence, and 4) assess effects of introduced fish on native macroinvertebrates. I identified two physiographic types of naturally fishless lakes in Maine: kettle lakes in the eastern lowlands and foothills and headwater lakes in the central and western mountains. Landscape-scale geomorphic and geographic factors correlated with fish absence were identified with GIS, and the likelihood that a particular lake is fishless was estimated with stepwise logistic regression. Regression models predicted that 4% (131) of 3281 lakes (0.6-10.1ha) in the two study regions were naturally fishless. Twenty-one lakes were

visited and sampled with gillnets and paleolimnological techniques to confirm current and historical fish absence, respectively. Models correctly predicted historical fish absence in 71% of the lakes, yet fish surveys indicated that many lakes now contain fish. Macroinvertebrates were sampled in 16 fishless and 18 fish-containing lakes to identify unique attributes of fishless lake communities. Macroinvertebrates in fishless lakes were more speciose and abundant, especially large, active and free-swimming taxa. *Graphoderus liberus*, *Hesperocorixa* spp., *Dineutus* spp., *Chaoborus americanus, Notonecta insulata* and *Callicorixa* spp. were identified as robust indicators of fish absence that were effectively collected with light traps. Fourteen historically fishless – now stocked – lakes were sampled to assess effects of introduced fish. Stocked lakes supported dramatically reduced macroinvertebrate abundance and species richness than currently fishless lakes. These effects were more pronounced in headwater than kettle lakes, likely due to sparse littoral habitat structure and intense stocking regimes. Maine's naturally fishless lakes provide habitat for a unique suite of organisms that thrive in the absence of fish predation. Fishless lakes warrant protection from fish introductions, and recovery of stocked fishless lakes will enhance conservation of this resource.

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1. INTRODUCTION

1.1 History of stocking fishless lakes

Beginning in the late 19th century and continuing through most of the 20th, game fish were introduced into numerous inland waters throughout North America to enhance recreational fishing. This was a time of anthropocentric natural resource management, when resources were managed for the benefit of people with little concern for ecological consequences (Stanley, 1995; Rahel, 1997; Pister, 2001). Native fish species, such as cyprinids (Whittier *et al.,* 1997), were displaced and previously fishless lakes and streams (Christenson, 1977) were stocked with species considered to be "desirable" game fish. At this time, humans undervalued the ecological importance of naturally fishless lakes and streams, viewing them as "barren" (Nilsson, 1972) or as "food for fishes… going to waste" (Schnitger, 1896). These habitats were valued primarily as potential sport fish habitat but also as bait fish rearing sites [e.g. prairie potholes (Hanson & Riggs, 1995)]. As a result, fish introductions led to a widespread decline in the number of fishless lakes, a phenomenon that has been documented worldwide. Fewer than 45% of 16,000 mountain lakes in the western United States remain unstocked, although 95% were naturally fishless (Bahls, 1992). At least 95% of the 1,464 lakes in western Canada's mountain national parks historically were naturally fishless, and 20% of these were altered through non-native fish introductions during the 20th century (Donald, 1987). Most mountain lakes in southeastern Norway were historically fishless, with >95% of the fish populations introduced into this region's lakes (Hesthagen & Sandlund, 2004).

The predominant geographical pattern of fish introductions in the United States has been westward movement of species native to eastern states, with only one western

game species [rainbow trout (*Oncorynchus mykiss*)] widely introduced in the East (Rahel, 2000). Despite this general pattern, New England states contain some of the most altered fish faunas in the USA due to the low number of native species considered desirable as game fish in this region (Whittier & Kincaid, 1999; Rahel, 2000). Fish have been moved liberally within their native New England ranges among lakes, with many instances of translocations of "native" fish to non-native waterbodies (Whittier & Kincaid, 1999). The state of Maine was an early leader in state-sanctioned game fish stocking and fish hatchery development. The first recorded attempt at game fish stocking in Maine occurred in 1868, with the introduction of landlocked Atlantic salmon (*Salmo salar*) into Cathance Lake (Warner & Havey, 1985). Soon after, the first public Atlantic salmon hatchery in the USA was created in Maine in 1871 (Moring, 2000). As fish culture techniques improved, salmon were introduced into many lakes in Maine and were distributed to at least 19 other states and several countries by the end of the 19th century (Warner & Havey, 1985; Moring, 2000). In addition to landlocked salmon, other native sport fish such as brook trout (*Salvelinus fontinalis* Mitchill) and lake trout (*Salvelinus namaycush* Walbaum in Artedi), and non-native species such as rainbow trout (*Oncorhynchus mykiss* Walbaum) and brown trout (*Salmo trutta* Linneaus), have been introduced to numerous lakes statewide (Halliwell, 2005). The state fisheries management program has documented the introduction of "native" brook trout to several lakes where fish populations historically were absent, and a program of regular stocking in these lakes continues today (MDIFW, unpublished).

1.2 Ecological value of fishless lakes and the effects of stocking

Naturally fishless lakes provide a unique habitat type along a freshwater habitat gradient of water permanency and predator transitions (Wellborn *et al.,* 1996). Many aquatic taxa segregate along gradients of hydroperiod and predation regimes (Werner & McPeek, 1994; Skelly, 1995; Wellborn *et al.*, 1996; Stoks & McPeek, 2003). Within such gradients permanent fishless lakes provide habitat for invertebrates and amphibians that are unable to withstand periodic drying and have evolved in the absence of fish predation (and thus are unable to coexist with fish). Because many freshwater taxa exhibit strong habitat-specific associations, the availability of alternative habitat types enhances regional species diversity (Stoks & McPeek, 2003). Additionally, fishless lakes provide important trophic habitat for migrating and breeding waterfowl (Eriksson, 1979; Hunter *et al.*, 1985; DesGranges & Rodrigue, 1986; McNicol *et al.,* 1987; Hanson & Riggs, 1995; Bouffard & Hanson, 1997) and prey items for passerines (P. Epanchin, unpublished) and reptiles (Matthews & Knapp, 2002).

Because organisms inhabiting fishless lakes have evolved in the absence of fish predation, their populations and communities are affected dramatically when fish are introduced (Nilsson, 1972; Lamontagne & Schindler, 1994; Leavitt *et al.*, 1994; Liss *et al.*, 1995; McPeek, 1998; Funk & Dunlap, 1999; Knapp *et al.*, 2005). Rarefaction of this unique habitat type due to widespread introductions of predatory fish has been linked to landscape-scale losses of native prey species, including zooplankton (Stoddard, 1987; Bradford *et al.*, 1998; Knapp *et al.,* 2001), amphibians (Bradford, Tabatabai & Graber, 1993; Fisher & Shaffer, 1996; Bradford *et al.*, 1998; Knapp *et al.*, 2001; Pilliod & Peterson, 2001; Denoel, Dzukic & Kalezic, 2005; Orizaola & Brana, 2006) and

macroinvertebrates (Bradford *et al.*, 1998; Carlisle & Hawkins, 1998; Knapp *et al.*, 2001). For example, widespread trout introductions into fishless lakes in the Sierra Nevada led to the fragmentation and decline of mountain yellow-legged frog (*Rana muscosa*) populations, now a federally listed species (Bradford *et al.*, 1993; Knapp & Matthews, 2000). While recent research has focused on direct predatory effects of introduced fish on native fishless lake taxa, unanticipated indirect effects also have been documented. Introducing fish into previously fishless lakes can disrupt in-lake ecosystem processes, such as such as nutrient cycling and primary productivity, by altering pelagic foodweb structure and increasing pelagic nutrient supply (Leavitt *et al.*, 1994; Schindler *et al.*, 2001). Fish effects can also extend into adjacent terrestrial systems through disruptions of trophic connections between aquatic and terrestrial food webs. For example, introduced fish exert indirect negative effects on adult amphibians that feed at lake margins (Finlay & Vredenburg, 2007) and on upland vertebrates, such as garter snakes (Matthews & Knapp, 2002; Knapp *et al.*, 2007) and passerine birds (P. Epanchin, unpublished), through competition for different life stages of lake-derived prey. Introduced fish may alter competitive relationships between terrestrial plants: fish reduce dragonfly abundances, which releases terrestrial invertebrate pollinators from adult dragonfly predation pressure, giving competitive advantage to insect pollinated plants (Knight *et al.*, 2005). A similar example of an unanticipated trophic cascade caused by introducing fish into fishless lakes is the indirect facilitation of invading bullfrogs in the Pacific Northwest: introduced fish increase bullfrog tadpole survival by reducing predatory dragonfly abundances (Adams *et al.* 2003).

1.3 Research justification and objectives

The beginning of the environmental movement in the 1960s led to a gradual paradigm shift towards biocentric natural resource management, and traditional fish stocking practices have come under question (Rahel, 1997; Pister, 2001). Growing concern over the effects of introduced fish on native fauna has stimulated recent research on fishless lakes, primarily in western North America (see above). This research has led to increased recognition of the ecological value of these systems and regional efforts to conserve and restore them (Milliron, 1999; Drake & Naiman, 2000; Donald *et al.*, 2001; Knapp *et al.*, 2001; Knapp *et al.*, 2005; Bunn *et al.*, 2007). Little is known about naturally fishless lakes and their associated communities in eastern North America, or the degree that these systems are affected by stocking.

Maine historically may have hosted many fishless lakes due to its recent (10,000 years before present) glacial history and resulting topography. This study was developed to gain a better understanding of the physical and biological attributes of fishless lakes in Maine, with the goal of providing the Maine Department of Inland Fisheries and Wildlife (MDIFW) information and tools to facilitate efficient and effective conservation planning for this resource. The goals of this research were: 1) develop a quantitative method to remotely detect naturally fishless lakes in Maine, 2) conduct a landscape-scale assessment of unique attributes of fishless lake macroinvertebrate communities, 3) identify macroinvertebrate bioindicators for efficient assessment of fish absence, and 4) assess the effects of fish introductions by studying invertebrate communities in historically fishless lakes.

Two physiographic types of naturally fishless lakes distributed across two biophysical regions in Maine were identified for study: kettle lakes in the eastern lowlands and foothills (Figure 1.1) and headwater lakes the central and western mountains (Figure 1.2). Fish have been absent naturally from these lakes since the last glaciation (10,000 years BP) created natural physical barriers to fish colonization (Schilling *et al.*, 2008*a*). Kettle lakes formed in depressions left by glacial ice blocks. Many kettles have no surface water connections to other waterbodies, and thus lack routes for fish movement. Additionally, many kettles are bog lakes with naturally low pH, which limits fish species richness (Rahel, 1984). In contrast, fishless lakes in western Maine are high altitude headwater cirques isolated from fish colonization by steep outlets impassable to fish.

Figure 1.1 – A fishless kettle lake in Maine's eastern lowlands and foothills.

Figure 1.2 – A fishless headwater lake in Maine's central and western mountains.

1.3.1 Historic distribution of fishless lakes

No estimates of the current or historical abundance and distribution of fishless lakes in the eastern United States exist. In order to conserve and restore fishless lakes, and to assess their overall importance to the biogeographic and ecological history of a given region, we must determine their historical distribution and identify lakes that remain fishless. Accurately estimating the distribution of fishless lakes prior to the widespread stocking efforts of the 20th century is difficult, however, because fish introductions have not always been well-documented. Previous estimates of fishless lake abundance and distribution elsewhere in North America and Europe have relied on information gathered by interviewing fisheries managers and biologists (Bahls, 1992; Hesthagen & Sandlund, 2004) and consulting fish stocking records (Donald, 1987). Estimates that rely on anecdotal and qualitative information about lake stocking history likely omit lakes with unknown or poorly documented fish stocking histories. The goal of the first chapter was to develop a quantitative method to remotely detect naturally fishless

lakes by addressing the following objectives: 1) use GIS to identify geomorphic and geographic variables associated with fish absence, 2) build a model based on these variables to predict the probability that a given lake is naturally fishless and 3) assess model accuracy and historical fish absence with fish surveys and paleolimnological techniques (Lamontagne & Schindler, 1994). This chapter provides a tool to managers to efficiently detect naturally fishless lakes, enabling targeted management and conservation activities in lakes with high probabilities of historical fish absence.

1.3.2 Unique attributes of fishless lake macroinvertebrate communities

Most ecological studies of fishless lakes in the East have been either taxon-specific (Bendell, 1986; Bennett & Streams, 1986; McPeek, 1990*a*; Stoks & McPeek, 2003; Strong & Robinson, 2004; Arnott & Jackson, 2006) or community-level studies of lakes within limited geographic regions (Bendell & McNicol, 1987; Brett, 1989; Bendell & McNicol, 1995). Additionally, most of these studies have focused on lakes that were not naturally fishless and that lost their fish populations due to acidification. A landscape-scale study of naturally fishless lakes is necessary to understand the potential effects of widespread fish introductions on native communities. The goal of the second chapter was to conduct a landscape-scale assessment of unique attributes of macroinvertebrate communities by addressing the following objectives: 1) characterize and compare macroinvertebrate communities in fishless headwater and kettle lakes, 2) identify unique attributes of fishless lake macroinvertebrate communities compared to lakes with fish, and 3) develop a method to efficiently identify fishless lakes when thorough fish surveys are not possible. This chapter provides a tool for managers to efficiently assess fish

absence in lakes that are remote, numerous, and difficult to thoroughly sample with traditional fish survey methods.

1.3.3 Effects of introduced fish in previously fishless lakes

Results from the first two chapters generated the objectives for the third chapter. Analyses of the historical distribution of fishless lakes in Maine indicated that many of these systems have been stocked (Schilling *et al.*, 2008*a*). Comparisons of macroinvertebrate communities between fishless lakes and similar lakes containing fish elucidated many unique attributes of fishless lake macroinvertebrate communities. Based on these results and on the well-documented effects of fish predation on macroinvertebrate communities (Macan, 1965; Pope *et al.*, 1973; Morin, 1984; Post & Cucin, 1984; Bendell & McNicol, 1987; Evans, 1989; Mallory *et al.*, 1994), the goal of the third chapter was to assess how native fishless lake macroinvertebrate communities are affected by introduced fish. The objectives of the third chapter were: 1) compare macroinvertebrate communities in currently fishless lakes to those in historically fishless (now stocked) lakes in each region, 2) assess whether the effects of introduced fish on macroinvertebrate communities differ between headwater and kettle fishless lakes, and 3) assess whether the effect of introduced fish on native macroinvertebrate communities varies with the amount of time lapsed since the original fish introduction.

2. PREDICTING THE LOCATIONS OF NATURALLY FISHLESS LAKES

2.1 Abstract

Fish have been introduced into many previously fishless lakes throughout North America over the past 100+ years. It is difficult to determine the historical distribution of fishless lakes, however, because these introductions have not always been well-documented. Due to its glacial history and low human population density, the state of Maine (USA) may host the greatest number of naturally fishless lakes in the northeastern United States. However, less than one quarter of Maine's 6000+ lakes have been surveyed for fish presence, and no accurate assessments of either the historical or current abundance and distribution of fishless lakes exist. We developed methods to assess the abundance and distribution of Maine's naturally fishless lakes (0.6 – 10.1 ha). We hypothesized that the historical distribution of fishless lakes across a landscape is controlled by geomorphic and geographic conditions. We used ArcGIS to identify landscape-scale geomorphic and geographic factors (e.g. connectivity, surrounding slope) correlated with fish absence in two geomorphic regions of Maine - the central and western mountains and the eastern lowlands and foothills. By using readily available GIS data our method was not limited to field-visited sites. We estimated the likelihood that a particular lake is fishless with a stepwise logistic regression model developed for each region. The absence of fish from western lakes is related to elevation (+), minimum percent slope in the 500m buffer (+), maximum percent slope in the 500m buffer (+) and percent cover of herbaceous-emergent wetland in 1000m buffer (-). The absence of fish from eastern lakes is related to the lack of a stream within 50m of the lake. The models predict that a total of 4% (131) of study lakes in the two regions were historically

fishless, with the eastern region hosting a greater proportion than the western region. We verified the model predictions with two complementary approaches. First we visited 21 lakes predicted to be fishless and assessed current fish presence with gillnetting. Second, we used paleolimnological techniques based on the abundance of *Chaoborus americanus* mandibles in the bottom segments of sediment cores. Fifteen of the 21 lakes predicted to be fishless currently contain fish. Paleolimnological evidence, however, suggests that ten of the 15 lakes were historically fishless and thus were subject to undocumented fish introductions. Our approach efficiently predicts the distribution Maine's naturally fishless lakes, and our results indicate that these habitats have declined due to fish introductions. Our method could be applied to other regions with similar geographic and geomorphic constraints on fish distributions as a tool to enhance conservation of a limited resource that provides habitat for unique biological communities.

2.2 Introduction

Throughout much of the 20th century, sport fish were introduced into numerous inland waters throughout North America to enhance recreational fishing (Pister, 2001). Native species, such as cyprinids, were displaced by “more desirable” sport fish (Whittier *et al.*, 1997), and in many cases previously fishless lakes and ponds were stocked (Christenson, 1977). Until recently, fishless lakes were considered to have limited value, indicated by their description as “barren” (Nilsson, 1972). Organisms inhabiting fishless lakes have evolved in the absence of fish predation; their populations and communities are affected dramatically when fish are introduced (Nilsson, 1972; Lamontagne & Schindler, 1994; Leavitt *et al.*, 1994; Liss *et al.*, 1995; McPeek, 1998; Funk & Dunlap, 1999; Knapp *et al.*, 2005). Fishless lakes provide habitat for amphibians (Funk & Dunlap,

1999; Knapp & Matthews, 2000; Denoel *et al.*, 2005), waterfowl (Bouffard & Hanson, 1997) and uniquely structured diatom (Drake & Naiman, 2000), zooplankton (McNaught *et al.*, 1999; Knapp *et al.*, 2001) and macroinvertebrate communities (McPeek, 1998; Schilling *et al.*, 2008*b*). Fishless lakes have been shown to enhance regional species diversity by providing a unique freshwater habitat type along a gradient of waterbody permanence and predator presence, ranging from temporary vernal pools lacking large dragonfly and fish predators to permanent lakes where fish are top predators (Stoks & McPeek, 2003). Efforts to conserve and restore fishless lakes recently have been stimulated by the recognition of their ecological value (Drake & Naiman, 2000; Donald *et al.*, 2001; Knapp *et al.*, 2001; Knapp *et al.*, 2005).

Fewer than 45% of 16,000 mountain lakes in the western United States remain unstocked, although 95% were naturally fishless (Bahls, 1992). At least 95% of the 1464 lakes in western Canada's mountain national parks historically were naturally fishless, and 20% of these were altered through non-native fish introductions during the 20th century (Donald, 1987). Most mountain lakes in southeastern Norway were historically fishless, with >95% of the fish populations introduced into this region's lakes (Hesthagen & Sandlund, 2004). Determining the distribution of fishless lakes prior to the widespread stocking efforts of the 20th century is difficult, because these fish introductions have not always been well-documented.

In order to conserve and restore fishless lakes, and to assess their overall importance to the biogeographic and ecological history of a given region, we must determine their historical distribution and identify lakes that remain fishless. The current and historical abundance and distribution of fishless lakes in the eastern United States is

unknown. Maine, in particular, historically may have hosted many fishless lakes due to its recent (10,000 years before present) glacial history and resulting topography. High elevation cirques in western Maine, for example, are isolated from fish colonization by steep outlets impassable to fish. In contrast, kettle lakes in eastern Maine have no surface water connections to other lakes and streams, and thus lack routes for fish movement. In addition, many eastern Maine lakes are bog lakes with naturally low pH, a factor limiting fish species richness (Rahel, 1984).

Stocking of lakes in Maine began in the late 1800s. Native sport fish such as landlocked Atlantic salmon (*Salmo salar* Linneaus), brook trout (*Salvelinus fontinalis* Mitchill) and lake trout (*Salvelinus namaycush* Walbaum in Artedi), and non-native species such as rainbow trout (*Oncorhynchus mykiss* Walbaum) and brown trout (*Salmo trutta* Linneaus), have been introduced to numerous lakes statewide (Halliwell, 2005). Although authorized fish introductions during the past 50 years are relatively well-documented, many lakes currently supporting fish populations likely were stocked prior to this time when introductions were poorly documented [Maine Department of Inland Fisheries and Wildlife (MDIFW), unpublished]. Although the state fisheries management program has documented the introduction of brook trout to several lakes where fish populations historically were absent (MDIFW, unpublished), the extent of undocumented introductions is unknown, as is the number of fishless lakes remaining. State fisheries biologists have conducted fish surveys in the majority of Maine's larger lakes, but < 40% of small lakes (< 40 ha) have been surveyed (Vaux, 2005). Considering that 81% of Maine's 9869 lakes are small (Vaux, 2005), it is likely that many of Maine's fishless lakes remain undocumented. Assessing the current status of fishless lakes in Maine is

important, because the proportion of historically fishless lakes in Maine that have been unaltered by stocking may be relatively high compared to elsewhere in the eastern United States due to the state's relatively low human population density. As a consequence, the state of Maine may provide an important landscape-scale refuge for aquatic biota that require fishless habitat.

We developed this study to facilitate conservation and restoration of these fishless habitats by identifying Maine's lakes that were likely naturally fishless. We hypothesized that the landscape-wide distribution of fishless lakes in Maine is controlled by geomorphic and geographic factors that can be assessed with remote sensing and geographic information systems (GIS). Our objectives were to: 1) use GIS to identify geomorphic and geographic variables associated with fish absence, 2) build a model based on these variables to predict the probability that a given lake is naturally fishless and 3) assess model accuracy and historical fish absence with fish surveys and paleolimnological techniques (Lamontagne & Schindler, 1994). Our goal was to facilitate conservation by developing a quantitative method with widely accessible GIS data to efficiently detect naturally fishless lakes, so that in-lake fish surveys could be targeted to lakes with or without fish.

2.3 Methods

2.3.1 Lake characterization

We selected lakes for model building with a combination of records from MDIFW (unpublished) and GIS-derived data describing lake physical characteristics. Fish species status and stocking history were determined from historical survey data and stocking records for 1940s to present (MDIFW, unpublished data). Two sets of lakes

were selected for model building: “naturally fishless” and “fish-containing.” Lakes were assumed to be naturally fishless if MDIFW surveys using gillnets and minnow traps did not capture fish during the 10 years preceding our study, or if the lake's historical status previously was documented as fishless prior to stocking. Fish-containing lakes were those for which MDIFW records did not indicate that the lake was historically fishless and where fish presence was documented. Lakes that had not been surveyed, lakes with unknown history and containing only stocked species and lakes with unconfirmed reports of fish presence were eliminated from the model-building dataset.

We identified our target lake population from a lake polygon coverage. We defined potentially fishless lakes as those water bodies with 0.6 - 10.1 ha surface area (the size of Maine's documented fishless lakes and minimum size detectible on Maine’s lake polygon coverage) and those located in the 10, eight-digit hydrologic unit code (HUC) catchments where fishless lakes have been documented (MDIFW, unpublished data). GIS (ArcGIS, version 8.3, ESRI Inc., Redlands, CA, USA) was used to describe geographic and geomorphic attributes of the selected lakes and their surrounding landscapes. Variables assessed for correlations with fish absence generally described the presence or absence of hydrological connectivity and other natural barriers to fish migration (Magnuson *et al.*, 1998; Hershey *et al.*, 1999; Hershey *et al.*, 2006). Variables selected from the Maine Lake Classification and Conservation Prioritization Project (A. Olivero, The Nature Conservancy, personal communication) and from our own GIS analyses were used for model building (Table 2.1). To characterize the landscape

surrounding lakes, several attributes (% wetland cover, stream density, % slope) were derived with GIS within 500m and 1000m buffers around the perimeter of each lake (Table 2.1). Due to gaps in the streams network data, streams within 50m of a lake were identified as connected to the lake. Other factors known to influence fish distributions, such as maximum lake depth (Tonn & Magnuson, 1982; Tonn *et al.*, 1990; Magnuson *et al.*, 1998; Jackson *et al.*, 2001), dissolved oxygen concentrations (Jackson *et al.*, 2001; Ohman *et al.*, 2006) and pH (Rahel, 1984; Magnuson *et al.*, 1998; Jackson *et al.*, 2001) were not included as model variables, because they could not be assessed remotely and no GIS coverages describing these parameters were available at the time of study.

2.3.2 Model building and assessment

We used multiple, stepwise logistic regression to predict the probability that a lake is fishless based on its geomorphic and geographic features (Table 2.1). Because logistic regression is sensitive to multicollinearity, we assessed all pairwise correlations between explanatory variables and based our regression models only on uncorrelated variables. Preliminary data analyses involved using univariate logistic regression to evaluate simple associations between fish absence and each independent variable. We then tested for multicollinearity between pairs of independent variables with multiple correlation analyses between continuous explanatory variables (Pearson correlation coefficient >0.7), multi-way frequency analysis between categorical variables (Pearson Chi-square, $P < 0.05$) and one-way analyses of variance between categorical and continuous variables (F-ratio, $P < 0.05$). Variables assessed within 500 and 1000m buffers were found to be collinear, and mean slope and maximum slope were collinear. In

these cases, the variable with the most explanatory power from preliminary univariate analyses was retained for use in stepwise logistic regression. No collinearity was found between the other explanatory variables.

Table 2.1 – Geomorphic and geographic variables used in logistic regression models predicting the fishless condition in Maine lakes (0.6 – 10.1 ha).

Variable	*GIS layer*	Data Source
Percent wetland cover by wetland type* (forested or herbaceous)	National landcover classification	The Nature Conservancy (TNC)
Stream density*	Streams network	current study
Minimum percent slope*	Digital elevation model	current study
Maximum percent slope*	Digital elevation model	current study
Mean percent slope*	Digital elevation model	current study
Presence of connecting stream (within 50m buffer around the perimeter of each lake)	Streams network	current study
Presence of connection to larger wetland complex	NWI	current study
Distance to nearest neighboring wetland (overland distance)	NWI	current study
Lake elevation	Digital elevation model	TNC
Distance to nearest neighbor wetland	NWI	current study
Dominant acid-neutralizing capacity class in 12-digit HUC catchment[†]	see footnote	TNC

* Variables assessed within 500 and 1000m buffers around the perimeter of each lake.

[†]Data in this layer were derived from bedrock and surficial geology GIS coverage for Maine. Bedrock geologic types were aggregated according the schema of Norton *et al.* (undated) and Norton (1980); types are classified via the extent to which they impart acid neutralizing capacity in surface waters. For the purposes of this study we examined relationships with dominant Norton class in the 12-digit HUC catchment of each lake.

Known fishless lakes in Maine are located primarily in two biophysical regions, the central and western mountains and the eastern lowlands and foothills (Krohn *et al.*, 1999, Figure 2.1). Different factors (e.g. topographical barriers in the west, hydrological barriers in the east) constrain fish distributions in these two regions due to their distinct geological and topographical characteristics; therefore, unique models were developed for western lakes and eastern lakes. All lakes (0.6 – 10.1 ha) in the selected catchments were entered into the model for their region.

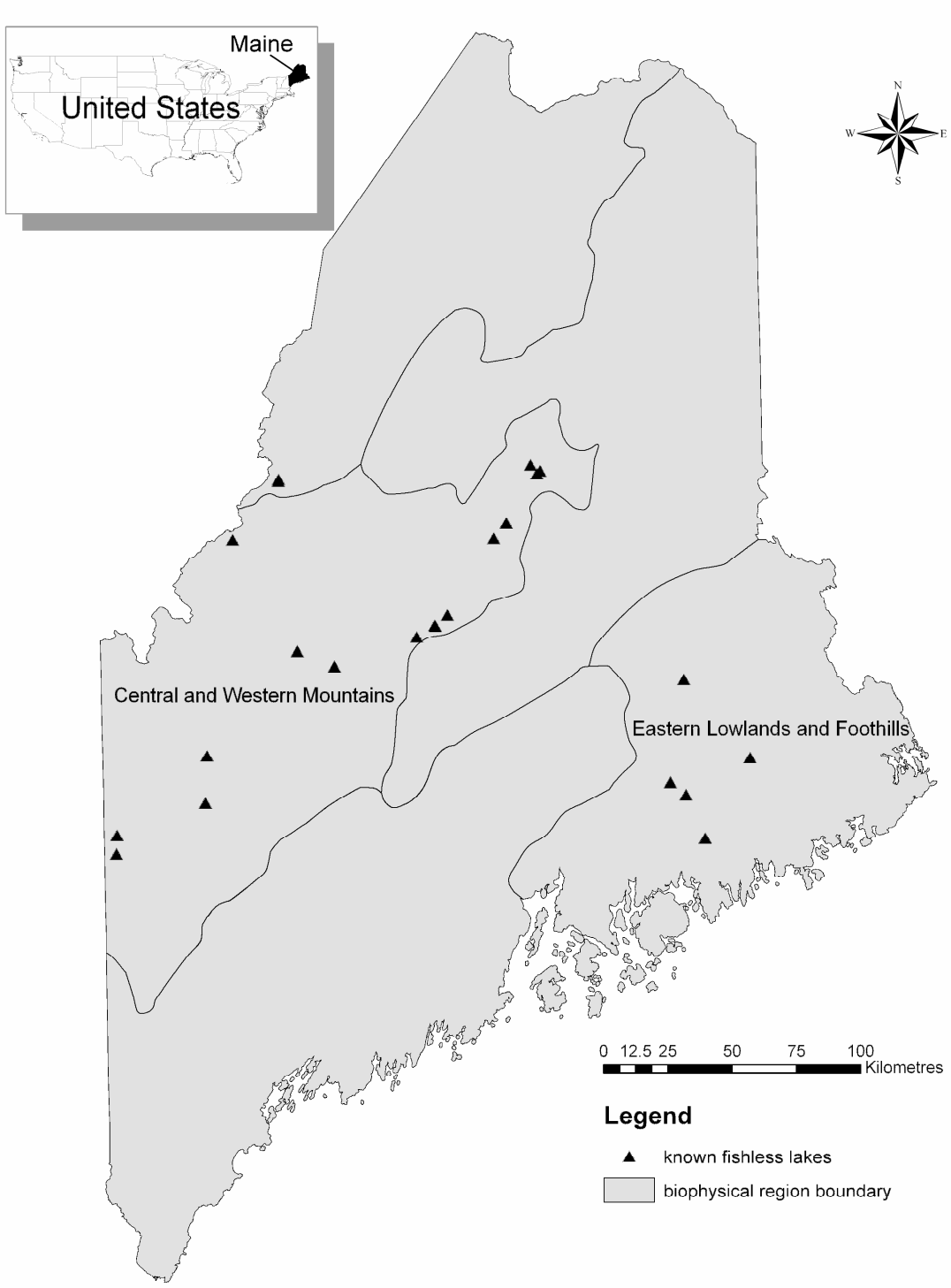

Figure 2.1 – Regions in Maine where known fishless lakes occur. Biophysical regions are delineated by Krohn *et al*. (1999).

Variables retained in the final model for each region were selected with forward stepwise selection with nominal cut-off at $P = 0.05$. Likelihood ratio tests were used to determine the significance of explanatory variables. Model significance and fit were assessed with McFadden's rho-squared (ρ^2) and the Hosmer-Lemeshow goodness of fit test. When multiple variables were retained in the final model, we performed a secondary test for multicollinearity by analyzing variance inflation (>10) and tolerance (<0.1). Model accuracy was evaluated by calculating the percentages of all cases correctly predicted (i.e. correct classification rate), cases correctly predicted in the response group (i.e. sensitivity) and cases correctly predicted in the reference group (i.e. specificity). Each regression equation resulted in a response value between 0 (low probability of fish absence) and 1 (high probability of fish absence). We concluded that lakes with probabilities ≥ 0.5 have a high probability of being fishless. We used SYSTAT (version 11, Systat Software Inc., Richmond, CA, USA) for all statistical analyses.

2.3.3 Model validation

A subset of 21 lakes (12 eastern, nine western) with ≥ 0.5 probability of being fishless was selected randomly for model validation. MDIFW fisheries data were reviewed to determine lake survey history. Lakes (n = 13) that had not been surveyed for fish recently (in the previous 10 years) were gillnetted and minnow trapped following MDIFW survey protocols (T. Obrey, MDIFW, personal communication). One monofilament gillnet (40m x 1.5m) containing four panels of 19mm, 25mm, 33mm and 38mm mesh was bottom-set perpendicular to shore for two, 15 minute sets and checked for fish between sets. If no fish were caught after two sets, the net was deployed

overnight and checked the following morning. Three minnow traps baited with dog biscuits were placed at equidistant intervals around the lake perimeter and checked for fish after 12 hours. Captured fish were identified and counted.

Fish occurrence in a lake predicted to be fishless indicates one of two conditions: 1) the GIS-based model does not accurately predict fish absence or 2) the model is valid and the lake was historically fishless, but undocumented fish stocking occurred. To determine which of these conditions was more likely, we developed a paleolimnological inference (PI) method calibrated for lakes in Maine (DeGoosh, 2007) to determine the probability that each lake in the GIS-based model validation sample was historically fishless.

The PI method uses mandibles of the phantom midge *Chaoborus americanus* (Johannsen) in lake sediments to indicate fish absence. Because *Chaoborus americanus* larvae are intolerant of fish predation and their mandibles are preserved in lake sediments for hundreds of years, they provide a good indicator of historical fish absence (Uutala, 1990; Lamontagne & Schindler, 1994; Sweetman & Smol, 2006). To test this as an indicator in Maine, *Chaoborus* mandibles were identified from sediments collected from a subset of known fishless lakes and fish-containing lakes. *Chaoborus americanus* was present in low numbers in some fish-containing lakes; therefore, we were unable to rely simply on the presence of *C. americanus* to indicate fish absence (DeGoosh, 2007). Instead, we used logistic regression to predict the probability a given lake was historically fishless based on the abundance of *C. americanus* mandibles relative to the abundance of all *Chaoborus* species in the sediment sample (DeGoosh, 2007). The probability that a

lake is historically fishless increases along a sigmoidal curve as the relative abundance of *C. americanus* increases (DeGoosh, 2007).

We used the PI method to assess our GIS-based model in lakes selected for model validation that were found to contain fish (Figure 2.2). We collected three sediment cores with a Hongve corer (15 cm long x 5 cm diameter) from the deepest location within the lake, and sectioned the cores at 0.5 centimeter increments in the field. We assumed that sediment taken from the bottom centimeter of the core predated fish stocking, based on sedimentation rates from cores collected from nearby lakes and dated with radioisotope Pb-210 (Davis *et al.*, 1994). We counted mandibles in the bottom sections of each core and identified them to species (Uutala, 1990). Mandible abundance of at least one mandible per 10 cm^3 of sediment is required for historical conditions to be appropriately assessed using the PI method (DeGoosh, 2007). We specified a probability threshold of $P \geq 0.5$ for classification of lakes as historically fishless. When the PI method predicted a $\geq$ 50% probability that a lake was historically fishless, suggesting that the GIS-based model prediction of "fishless" was valid, we classified the lake as historically fishless and assumed undocumented fish stocking had occurred.

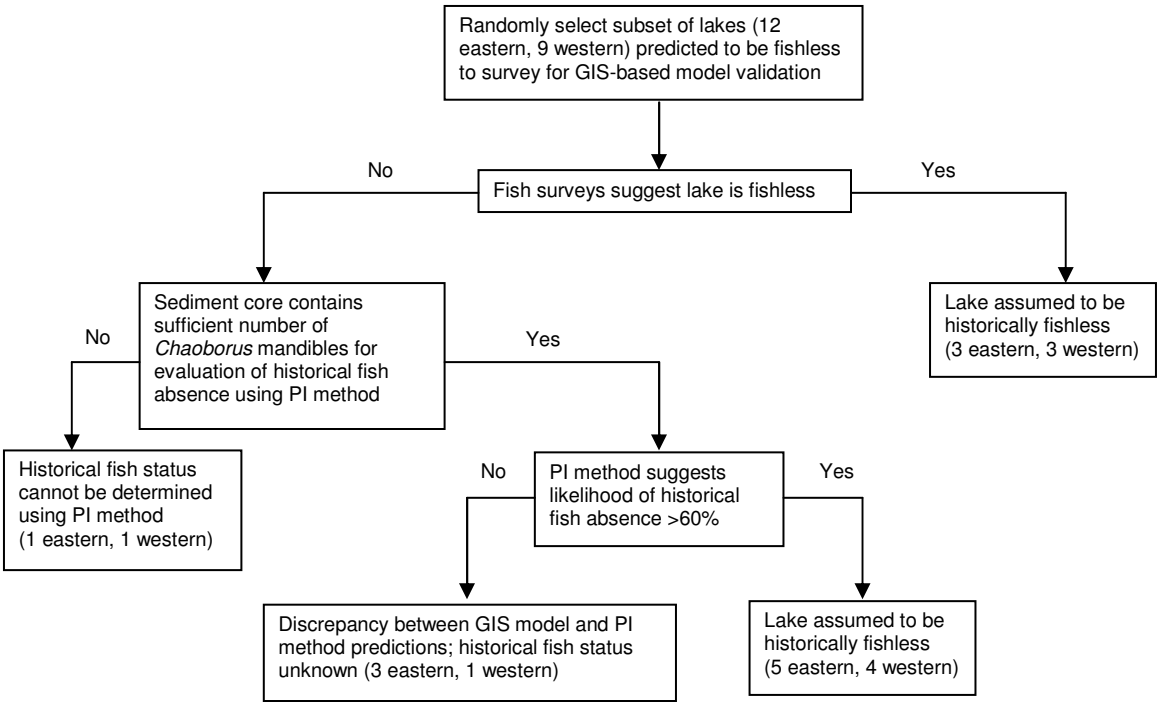

Figure 2.2 – Decision tree depicting the GIS-based model validation process.

2.4 Results

2.4.1 Model development

We identified 2514 lakes within the study size range in the central and western mountains. Complete fisheries records providing data required for model building were available for 302 of these lakes (278 fish-containing, 24 fishless, Table 2.2). We identified 767 lakes in the eastern lowlands and foothills. Complete fisheries data were available for 55 of these lakes (49 fish-containing, six fishless, Table 2.2).

Table 2.2 – Numbers of lakes (0.6 – 10.1 ha) used for building models to predict fishless lakes in the central and western mountains and eastern lowlands and foothills of Maine.

Region	*# of lakes*	*# known fishless lakes used for model building*	*# fish-containing lakes used for model building*	*# of additional lakes predicted to be fishless*	*% of lakes known and predicted to be fishless*
Central and Western Mountains	2514	24	278	33	2.3
Eastern Lowlands and Foothills	767	6	69	68	9.6
Total	3281	30	347	101	4.0

The absence of fish from eastern lakes is related to the lack of a stream within 50m of the lake (Fisher exact test: n = 55, P<0.001). Five of six known fishless lakes in the eastern lowlands and foothills have no stream connections, whereas all of the eastern fish-containing lakes have streams present within a 50m buffer. Because stream connectivity is a binary variable, logistic regression is not needed to characterize the likelihood of fish absence. For simplicity, we identified lakes in this region lacking stream connections within a 50m buffer and categorized them as having ≥50% probability of being historically fishless. One known fishless lake in this region, Mud Pond, is connected to streams, but is thought to be fishless due to a naturally low pH (Davis *et al.*, 1994).

The absence of fish from western lakes was related to elevation (+), minimum percent slope in the 500m buffer (+), maximum percent slope in the 500m buffer (+) and percent cover of herbaceous-emergent wetland in 1000m buffer (-) (Table 2.3). These characteristics differed between fishless and fish-containing western lakes (Figure 2.3). The likelihood-ratio statistic indicated that at least one predictor had a significant effect (2*(LL(N)-LL(0)) = 73.938, df = 4, χ^2 P-value < 0.0001), and P-values associated with

each variable were significant ($\alpha = 0.05$). Model fit was good ($\rho^2 = 0.441$; $\chi^2 = 1.443$, df = 2, P = 0.486), with a correct total classification rate = 0.950 (287 of 302), model sensitivity = 0.541 (13 of 24) and model specificity = 0.986 (274 of 278). Variance inflation factors and tolerance values indicated that multicollinearity between explanatory values was not significant (Table 2.3).

Together the models predicted that 101 lakes (33 western, 68 eastern) have ≥ 50% probability of being fishless in the two study regions, in addition to the 30 known fishless lakes used for model building (Table 2.2; Figure 2.4). Therefore, we predicted that of the 3281 eastern and western lakes we identified in the 0.6 – 10.1 ha size range, 4.0% are naturally fishless. The proportion of lakes predicted to be fishless is greater in the eastern region (9.6%) than in the western region (2.3%).

Table 2.3 – Logistic regression model predicting probability of fish absence in western Maine lakes.

Variable	*Estimate*	*SE*	*t-ratio*	*P-value*	*Variance Inflation Factor*	*Tolerance*
Constant	-5.444	1.026	-5.305	0.000	na	na
Altitude	0.005	0.002	2.455	0.014	1.513	0.661
Minimum slope (%) in 500 m buffer	1.828	0.688	2.656	0.008	1.365	0.732
Maximum slope (%) in 500 m buffer	0.026	0.013	2.012	0.044	1.437	0.696
Herbaceous wetland (%) in 1000 m buffer	-3.144	1.544	-2.037	0.042	1.193	0.838

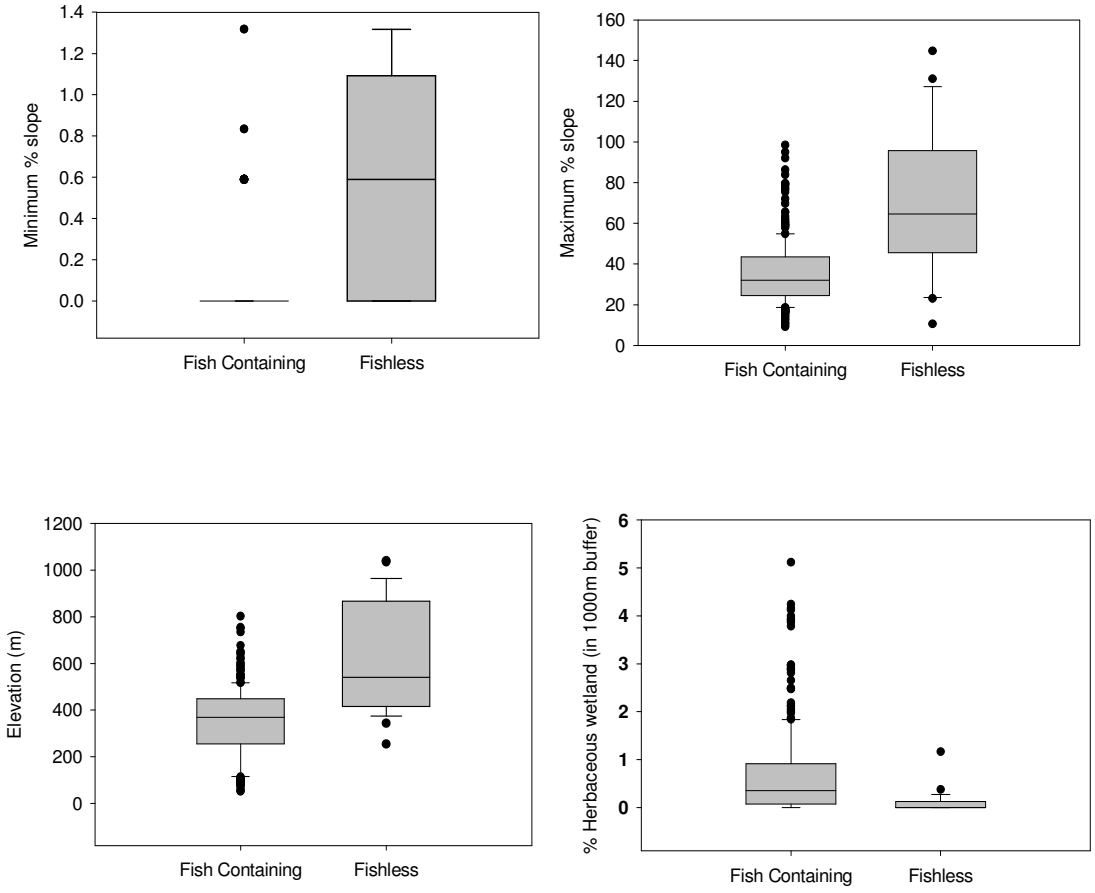

Figure 2.3 – Box plots for fish-containing and fishless lakes in western Maine showing dependent variables identified by stepwise logistic regression. Boxes indicate the 25th and 75th percentiles. The line within each box indicates the median.

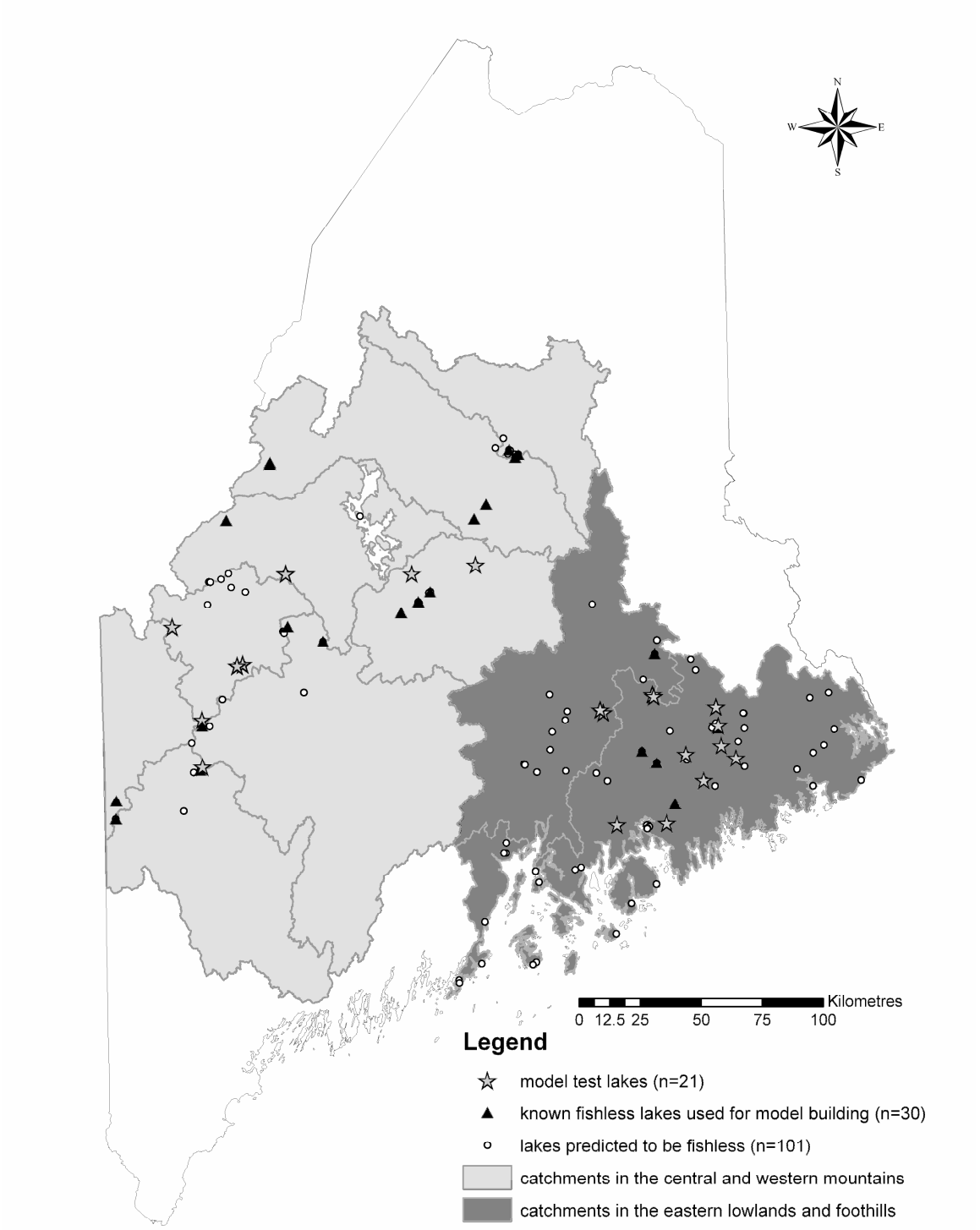

Figure 2.4. Geographic distribution of predicted fishless lakes and lakes used for model validation in Maine.

2.4.2 Model validation

Based on fish surveys, fifteen (nine eastern, six western) of 21 lakes of the model-validation sample contain fish and six (three eastern, three western) lakes are currently fishless. Paleolimnological results gave a ≥50% probability that 10 (six eastern, four western) of the 15 lakes that currently contain fish were historically fishless (Table 2.4). One eastern lake surveyed to validate the predictive GIS-based model lacked *Chaoborus* spp. mandibles, so historical fish absence could not be assessed with the PI method. Combining results from fish surveys and the PI method, the GIS models accurately predicted the fish status of 16 (nine eastern, seven western) of 21 model validation lakes. Fish communities currently found in historically fishless lakes vary by region. Fish assemblages in eastern lakes are more diverse, with most lakes containing brook trout and at least one minnow species, whereas western lakes generally contain only brook trout (Table 2.4). Brook trout are stocked regularly in seven lakes (five eastern, two western) of the model-validation sample, and two lakes contained illegally introduced golden shiners, a common bait fish. All fish species documented in these lakes, except for smallmouth bass (*Micropterus dolomieu* Lacepède), are native to Maine but not necessarily to these waters.

Table 2.4 – Predicted probabilities of current and historical fish absence in eastern and western model validation lakes. GIS-based model probabilities were estimated for eastern lakes and were calculated using logistic regression for western lakes. Probability of historical fish absence was calculated using the PI method. Current fish species composition identified in surveys is indicated; currently fishless lakes were not evaluated with the PI method. Rows highlighted in grey indicate lakes where fish survey data or PI method confirms GIS-based model prediction of fish absence.

Lake name	*Region*	*GIS-based model probability of fish absence*	*PI method prediction of historical fish absence*	*Current fish species composition*[a]
Black Brook #4	East	≥ 0.5	not evaluated[b]	fishless
Oak	East	≥ 0.5	not evaluated[b]	fishless
Unnamed 9629	East	≥ 0.5	not evaluated[b]	fishless
Crystal	East	≥ 0.5	fish-containing	brook trout*, fathead minnow, golden shiner
Pineo	East	≥ 0.5	fish-containing	brook trout*, golden shiner†, three-spined stickleback
Loon	East	≥ 0.5	fish-containing	brook trout, golden shiner
Salmon	East	≥ 0.5	fishless	brook trout*, fathead minnow, golden shiner
Pickerel	East	≥ 0.5	fishless	brook trout*, brown bullhead, chain pickerel, golden shiner
Crocker	East	≥ 0.5	fishless	fathead minnow, golden shiner
Unnamed 7537	East	≥ 0.5	fishless	pumpkinseed sunfish
Simmons	East	≥ 0.5	fishless	brook trout*, brown bullhead, golden shiner†, smallmouth bass, white sucker
Unnamed 8417	East	≥ 0.5	fishless	golden shiner
Cranberry	West	0.828	not evaluated[b]	fishless
Crater	West	0.948	not evaluated[b]	fishless
Jackson	West	0.974	not evaluated[b]	fishless
Rock	West	0.714	fish-containing	brook trout*
Horns	West	0.989	fishless	brook trout*
Notch	West	0.614	fishless	brook trout
Snow Mountain	West	0.950	fishless	brook trout
Mountain #1	West	0.915	fishless	brook trout
Mountain #2	West	0.868	not evaluated[c]	brook trout

[a] Introduced species are indicated by * (legal) and † (illegal).
[b] Lake currently fishless, therefore, not evaluated with PI method.
[c] Insufficient mandible abundance to evaluate the probability of fish absence based on the PI method.

2.5 Discussion

2.5.1 Eastern lake predictions and model assessment

Small lakes in the eastern lowlands and foothills of Maine tend to be hydrologically isolated kettle lakes lacking stream connections. Hydrological connectivity in the form of inlet and outlet streams is positively correlated with fish species richness in Wisconsin, presumably because these allow routes for colonization and refuge from predators (Rahel, 1984; Magnuson *et al.*, 1998). Hydrological isolation also appears to be a critical factor determining the distribution of fish in eastern Maine; the absence of streams within 50m characterizes five of six known fishless lakes in eastern Maine. Based upon this single variable, we predicted that ~10% (n = 74) of all 0.6 - 10.1 ha lakes in the region were historically fishless.

It is important to recognize, however, that hydrological connectivity likely is not the only variable determining the distribution of fish in eastern Maine. Acidity also is likely a critical factor; these kettle lakes share characteristics with Wisconsin bog lakes, where low fish species richness was correlated with low pH, low structural heterogeneity and lack of stream connections (Rahel, 1984). Because pH cannot be assessed remotely, it is not used as a predictor of fish absence in our study. We attempted to account for pH in our GIS model development by assessing the dominant acid neutralizing capacity (ANC) class (Norton, 1980) at the catchment scale. This variable was not significant in our stepwise model, most likely because the GIS data layer represents fairly broad classes of ANC and heterogeneity within a catchment can be substantial, and the spatial scale was too coarse to represent the within-catchment heterogeneity. Nonetheless, there likely are lakes with sufficient stream connectivity to allow fish colonization that did not

historically contain fish because of naturally low pH (e.g. Mud Pond). GIS-based variables that are correlated with specific geochemical attributes (i.e. vegetation and ANC) and that are assessed at a scale that captures the within-catchment geological heterogeneity may identify lakes that are fishless due to conditions such as low pH, and therefore provide a less conservative estimate of the number of fishless lakes in Maine's eastern lowlands and foothills.

Three of 11 lakes in the eastern model-validation sample from which *Chaoborus* mandibles were recovered were incorrectly predicted to be fishless with our GIS-based approach. We attributed one of these prediction errors to GIS data quality problems created by an incomplete streams network coverage, which emphasizes the importance of ground-truthing for producing accurate GIS-based analyses. Although the remaining two misclassified lakes lack extant stream connections, they may have been colonized via historical stream connections that no longer exist (Hershey *et al*., 1999). It also is possible that these two lakes were historically fishless, but fish absence is not reflected in the assemblage of *Chaoborus* spp. mandibles preserved in sediment cores. Although cores collected from these lakes met the minimum detection criterion (i.e. ≥ 1 *Chaoborus* mandible per 10 cm^3 of sediment) for the PI method, these cores contained low numbers of *Chaoborus* mandibles relative to other lakes in the region. The reasons for these low abundances are unknown but may reflect high sedimentation rates, patchy mandible distribution in the sediment or simply low *Chaoborus* abundance (Uutala & Smol, 1996).

2.5.2 Western lake predictions and model assessment

Unlike the eastern lowland and foothill lakes of Maine, the central and western lakes are high elevation cirques (254 – 1039 m a.s.l.) with steep outflowing streams.

Patterns of fish distribution are related to the gradient of these outlet streams. Stream gradient is related to fish presence in other mountainous regions [e.g. Alaska (Hershey *et al.,* 1999), Finland (Tonn *et al.,* 1990)], and other studies in northeastern North America have shown negative correlation between lake elevation and native and introduced fish species richness (Whittier & Kincaid, 1999). In general, mountain lakes lacking fish tend to be located in small, high-elevation catchments and have basins surrounded by steep slopes that are likely to have steep outflowing streams with waterfalls that prevent upstream fish migration. In our study, however, the probability of these lakes being fishless is shown to be not only positively related to lake elevation and maximum and minimum slopes within 500m around the lake, but also negatively related to percent herbaceous wetland cover within 1000m. The negative relationship between percent herbaceous wetland cover within 1000m and fish absence indicates that, similar to eastern lakes, western fishless lakes exhibit some degree of hydrological isolation. In western lakes this isolation is due to disjunction from wetlands, whereas, in eastern lakes this isolation is due to distance from streams. Based upon lake elevation, catchment slope attributes and proportion of wetland cover, we predict that ~2% (n = 57) of all 0.6 - 10.1 ha lakes in the mountains of central and western Maine were historically fishless.

One of the nine lakes in the western model-validation sample from which *Chaoborus* mandibles were recovered was incorrectly predicted to be fishless. According to our GIS-based model, this lake has a 71% probability of being naturally fishless based on its landscape setting. The PI method, however, indicates a low probability that this lake was historically fishless. Although this lake is in a catchment with steep slopes, the gradient of its outlet is moderate and presumably allows colonization of the lake by fish.

A measure of actual stream slope rather than slope within a buffer obviously would be more accurate. The resolution of the streams network coverage available at the time of study was too coarse to provide this information.

2.5.3 Benefits and limitations of using GIS to predict fishless lake locations

Previous estimates of fishless lake abundance and distribution have been based on qualitative information gathered by interviewing fisheries managers and biologists (Bahls, 1992; Hesthagen & Sandlund, 2004) and consulting fish stocking records (Donald, 1987). Our quantitative approach is more efficient and comprehensive than relying on anecdotal and qualitative information on lake stocking history, because it allows assessment of lakes with unknown or poorly documented fish stocking histories. Other studies have predicted fish presence and species distributions by quantifying lake and landscape features from topographic maps. For example, potentially fishless lakes in western Maine were identified from USGS quadrangles by locating lakes with steep, outflowing streams (Obrey, 2002). Magnuson *et al.* (1998) used topographic maps to manually assess variables, such as outflowing stream slope and presence of stream connections, in a multivariate approach to predict fish species richness and composition in lakes in Wisconsin and Finland. Their models also included within-lake characteristics such as maximum depth, pH and conductivity. Our GIS-based approach is more efficient than lake-by-lake manual map assessments, because it allows parameter assessment for many lakes simultaneously over broad geographic areas. Additionally, our method allows assessment of lakes that have never been visited, because it does not depend on knowledge of within-lake characteristics. This is an important consideration in lake-rich regions such as Maine, where the majority of small lakes have never been surveyed.

Hershey *et al.* (1999) used LIDAR (airborne laser rangefinder coupled to global positioning sensors) to measure outflowing stream gradient in arctic lakes, which was one of several variables included in their model describing landscape control on fish distribution in arctic lakes. Although LIDAR provides a more precise measurement of stream slope than our approximate measure of surrounding slope, analysis of pre-existing GIS data layers is more cost effective than generating location specific data with such remote sensing techniques. Ideally, a greater resolution stream network data layer would improve accuracy of outflowing stream presence and slope and would enhance GIS-based model prediction accuracy where stream connectivity affects fish presence.

There are limitations of relying exclusively on variables readily available in GIS data layers to predict fish absence; within-lake conditions that may be important predictors of fish presence are not included in our models. Some of the lakes identified as likely inhabited by fish due to lack of physical barriers to colonization actually may be fishless due to low pH, lack of significant habitat structure or water depths allowing complete freezing during winter. Therefore, our model predictions that naturally fishless lakes in Maine were historically rare and are a small fraction of the state's lakes (4% of 3281 study lakes) should be considered conservative. Our estimates of fishless lakes in western Maine also are affected by bias in logistic regression that favors classification into the larger group defined by the dependent variable (Hosmer & Lemeshow, 1989). In our analysis there were more records of fish presence than fish absence in western Maine, therefore our model is more likely to predict that a particular lake contains fish rather than is fishless. We also must caution that our method for validating GIS-based model predictions with the PI method is compromised by additive error. The PI method

indicated that 10 of 14 predicted lakes were fishless with a 16% error rate (DeGoosh, 2007). Information on historical fish status in most of these lakes is not available, so we must rely on inferential methods to estimate the likelihood that lakes now containing fish were historically fishless.

In spite of the limitations of our approach, we believe that GIS-based models provide a more efficient and cost-effective means of identifying potentially fishless lakes than manual assessment and anecdotal methods employed by previous studies. Additionally, GIS data layers are available in many regions, making our approach applicable where fish presence is constrained by similar geomorphic variables. Our method would be particularly valuable in regions where management and conservation of native communities is a priority, such as in western USA National Parks and wilderness areas. The National Park Service and the California Department of Fish and Game have begun restoration efforts in historically fishless lakes in the Sierra Nevada to reduce fragmentation of mountain yellow-legged frog (*Rana muscosa* Camp) populations, an endangered species (Milliron, 1999; Bunn *et al.*, 2007; Yosemite National Park, 2006). We know of two studies that assessed the historical number and distribution of fishless lakes in this region; these assessments were based primarily on interviews with fisheries managers (Bahls, 1992; Knapp, 1996). A quantitative analysis of historical fishless lake distributions in this region could be used to verify anecdotal evidence of historical fish absence in order to facilitate restoration efforts.

Another interesting application of our method would be to assess historical distributions of naturally fishless lakes in acidified regions such as the Adirondacks region of northern New York, USA, where fishless lakes are prevalent at high elevations

(Jenkins *et al*., 2005). Some of these lakes historically supported fish populations and have lost them due to acidification; however, some of these lakes are naturally fishless due to barriers to upstream fish migration (similar to fishless lakes in western Maine). The Adirondacks region also hosts naturally fishless seepage lakes with no inlets or outlets (similar to fishless lakes in eastern Maine). There is a significant correlation between acidity and fish absence in this region (Jenkins *et al*., 2005); however, it is important to not assume that all acidified fishless lakes have lost their fish populations. Naturally fishless lakes fall into two classes of lakes in the region that are most susceptible to acidification: high elevation drainage lakes and lowland seepage lakes (Jenkins *et al*., 2005). Therefore, it is difficult to separate those that are naturally fishless from those that have lost fish populations, because both lake types may be acidified. Our GIS-based approach combined with paleolimnological methods could be used to assess which lakes were naturally fishless so that reclamation efforts can be concentrated on lakes that have lost their fish populations and to avoid re-introduction of fish into lakes that were historically fishless.

2.5.4 Management implications

Fishless lakes support aquatic communities that vary markedly from those of lakes containing fish (Stoks & McPeek, 2003; Schilling *et al.*, 2008*b*), potentially serve as refuges for genetically unique populations, particularly for taxa with low vagility, such as calanoid copepods (McNaught *et al.*, 1999; Knapp *et al.*, 2001) and provide critical breeding habitat for amphibians (Funk & Dunlap, 1999; Knapp & Matthews, 2000; Denoel *et al.*, 2005). Our results indicate that this habitat-type in Maine has declined during the last two centuries due to introductions of several fish species, with brook trout

the most widely distributed species in these habitats. Given the declining number of these habitats throughout Maine, North America and elsewhere (Donald, 1987; Bahls, 1992; Denoel *et al.*, 2005), their rarity and role in maintaining biodiversity, historically fishless lakes should be prioritized for conservation. GIS-based models such as ours can identify lakes that are currently fishless to target them as conservation priorities, and to identify lakes currently containing fish that were likely historically fishless as potential sites for restoration to the natural fishless condition. While ground-truthing is necessary to determine the current and historical status of fish in each lake, our approach allows managers to efficiently apply their resources to identify these habitats and target their management and conservation activities in lakes with high probabilities of historical fish absence.

2.6 Chapter acknowledgments

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3. MACROINVERTEBRATES AS INDICATORS OF FISH ABSENCE IN NATURALLY FISHLESS LAKES

3.1 Abstract

Little is known about native communities in naturally fishless lakes in eastern North America, a region where fish stocking has led to a decline in these habitats. Our study objectives were to: 1) characterize and compare macroinvertebrate communities in fishless lakes found in two biophysical regions of Maine (USA): kettle lakes in the eastern lowlands and foothills and headwater lakes in the central and western mountains, 2) identify unique attributes of fishless lake macroinvertebrate communities compared to lakes with fish and 3) develop a method to efficiently identify fishless lakes when thorough fish surveys are not possible. We quantified macroinvertebrate community structure in the two physiographic fishless lake types (n = 8 kettle lakes; n = 8 headwater lakes) with submerged light traps and sweep nets. We used non-metric multidimensional scaling to assess differences in community structure and *t*-tests for taxon-specific comparisons between fishless kettle and headwater lakes. We also compared fishless lake macroinvertebrate communities to those in fish-containing lakes (n = 18) of similar size, location and maximum depth. We found few differences in macroinvertebrate communities between the two physiographic fishless lake types. Fishless and fish-containing lakes had numerous differences in macroinvertebrate community structure, abundance, taxonomic composition and species richness. Fish presence or absence was a stronger determinant of community structure in our study than differences in physical conditions relating to lake origin and physiography. Communities in fishless lakes were more speciose and abundant than in fish-containing lakes, especially taxa that are large,

active and free-swimming. Families differing in abundance and taxonomic composition included Notonectidae, Corixidae, Gyrinidae, Dytiscidae, Aeshnidae, Libellulidae and Chaoboridae. We identified six taxa unique to fishless lakes that are robust indicators of fish absence: *Graphoderus liberus*, *Hesperocorixa* spp.*, Dineutus* spp.*, Chaoborus americanus, Notonecta insulata* and *Callicorixa* spp.. These taxa are collected most effectively with submerged light traps. Naturally fishless lakes warrant conservation, because they provide habitat for a unique suite of organisms that thrive in the absence of fish predation.

3.2 Introduction

Historically, the unique biological communities inhabiting fishless lakes have been undervalued. During the past two centuries these lakes were viewed primarily as potential habitat for sport fish, resulting in widespread fish introductions into fishless lakes with little regard for effects on native species (Donald, 1987; Bahls, 1992; Pister, 2001). This habitat type was common, especially at high altitudes, but extensive fish stocking has decreased the number of fishless lakes worldwide. For example, fewer than 45% of 16,000 mountain lakes in the western United States remain unstocked, although 95% were naturally fishless (Bahls, 1992). At least 95% of the 1,464 lakes in western Canada's mountain national parks were naturally fishless; 20% of these have been altered through non-native fish introductions during the 20th century (Donald, 1987). Historically, these habitats probably were less common in eastern North America where topographic relief is lower, but abundance of this habitat type in this region has declined as a result of fish stocking as well (Schilling *et al.*, 2008*a*).

Fishless lakes provide a unique habitat type along a freshwater habitat gradient of water permanency and predator transitions (Wellborn *et al.*, 1996). Many aquatic taxa are known to segregate along gradients of hydroperiod and predation regimes (Werner & McPeek, 1994; Skelly, 1995; Wellborn *et al.*, 1996; Stoks & McPeek, 2003). Within such gradients permanent fishless lakes provide habitat for invertebrates and amphibians that are unable to withstand periodic drying and have evolved in the absence of fish predation (and thus are unable to coexist with fish). Because many freshwater taxa exhibit strong habitat-specific associations, the availability of alternative habitat types enhances regional species diversity (Stoks & McPeek, 2003). Widespread fish introductions into permanent fishless lakes have effectively removed this unique habitat type from some regions, leading to dramatic declines of native taxa in the landscape (Bradford *et al.*, 1993; Pilliod & Peterson, 2001; Denoel *et al.*, 2005). For example, widespread trout introductions into fishless lakes in the Sierra Nevada led to the fragmentation and decline of mountain yellow-legged frog (*Rana muscosa*) populations, now a federally listed species (Bradford *et al.*, 1993; Knapp & Matthews, 2000). Recognition of the deleterious effects of non-native fish has led to conservation and restoration of these increasingly rare habitats, particularly in the western USA (Milliron, 1999; Yosemite National Park, 2006; Bunn *et al.*, 2007).

Although communities inhabiting naturally fishless lakes in western North America have been the focus of recent research (e.g. Knapp *et al.,* 2005), those in eastern North America have been little studied. Most studies of fishless lakes in this region have been taxon-specific (Bendell, 1986; Bennett & Streams, 1986; McPeek, 1990*a*; Stoks & McPeek, 2003; Strong & Robinson, 2004; Arnott & Jackson, 2006) and have focused on

acidified lakes that have lost their fish populations (Bendell, 1986; Bendell & McNicol, 1987; Bendell & McNicol, 1995; Strong & Robinson, 2004; Arnott & Jackson, 2006). Communities inhabiting different physiographic types of naturally fishless lakes may be dissimilar. Understanding these differences and how fishless lake communities compare to those with fish may reveal aspects that potentially are lost with fish introduction. Our study compares macroinvertebrate communities in two types of naturally fishless lakes distributed across two biophysical regions in Maine: kettle lakes in the eastern lowlands and foothills and headwater lakes the central and western mountains (Schilling *et al.*, 2008*a*). Fish have been naturally absent from these lakes since the last glaciation (10,000 years BP) created natural physical barriers to fish colonization. Fishless lakes in eastern Maine are kettle lakes formed in depressions left by glacial ice blocks. Many kettles have no surface water connections to other waterbodies, and thus lack routes for fish movement. Additionally, many kettles are bog lakes with naturally low pH, which limits fish species richness (Rahel, 1984). In contrast, fishless lakes in western Maine are high altitude headwater cirques isolated from fish colonisation by steep outlets impassable to fish.

The goal of our study is to inform management of these lakes by addressing three objectives: 1) characterize and compare macroinvertebrate communities in the two physiographic fishless lake types, 2) identify unique attributes of fishless lake macroinvertebrate communities compared to lakes with fish, and 3) develop a method to efficiently identify fishless lakes when thorough fish surveys are not possible. Addressing the first objective, we hypothesized that macroinvertebrate communities would differ between fishless kettle lakes in eastern Maine and fishless headwater lakes in western

Maine due to contrasting lake physical conditions and landscape setting (Krohn *et al.*, 1999; Schilling *et al.*, 2008*a*). Addressing the second objective, we hypothesized that due to known effects of fish predation on macroinvertebrate communities (Macan, 1965; Pope *et al.*, 1973; Morin, 1984; Post & Cucin, 1984; Bendell & McNicol, 1987; Evans, 1989; Mallory *et al.*, 1994), macroinvertebrate communities in fishless lakes would be distinct from similar lakes containing fish. Addressing the third objective, we recognize that time-and resource-limited managers need a means for efficiently assessing fish absence when lakes are remote, numerous, and difficult to thoroughly sample with traditional fish survey methods. We hypothesized that macroinvertebrate taxa exhibiting high affinity for fishless lakes would be useful bioindicators of the fishless condition, which could be efficiently assessed with an appropriate sampling technique.

3.3 Methods

3.3.1 Study lake selection and characterization

We identified 21 lakes (12 kettle lakes in the eastern lowlands and foothills, nine headwater lakes in the central and western mountains) that were likely to be fishless [Phillip deMaynadier, Maine Department of Inland Fisheries and Wildlife (MDIFW); Schilling *et al.*, 2008*a*]. We surveyed the lakes for fish with gillnets and minnow traps following MDIFW fish survey protocols (Tim Obrey, MDIFW, personal communication). One four-panel (19mm, 25mm, 33mm, 38mm mesh) monofilament gillnet (40m x 1.5m) was bottom-set perpendicular to shore for two, 15 minute sets and checked for fish between sets. If no fish were caught after two sets, the net was deployed overnight and checked the following morning. Three minnow traps baited with dog biscuits were placed at equal distances around the lake perimeter and checked for fish

after 12 hours. If no fish were captured during this survey, we considered the lake to be fishless. No fish were captured in 16 (eight kettle, eight headwater) lakes, and these were chosen as our fishless study lakes (Figure 3.1). We determined lake surface area, altitude and maximum depth with geographic information systems (GIS) and MDIFW records and collected one closed cell pH sample/lake (Table A.1). We compared these attributes between fishless kettle and headwater lakes with Student's *t*-tests ($\alpha = 0.1$). We qualitatively assessed habitat complexity (approximate amount of lake perimeter rimmed with littoral vegetation and distance it extended from shore) in the field.

We identified 18 fish-containing lakes (10 in eastern lowlands and foothills, eight in central and western mountains; Table A.1) for study (Figure 3.1). When selecting fish-containing lakes we minimized differences between fishless and fish-containing lakes in physical conditions (location, position in watershed, surface area, altitude, maximum depth) determined with GIS and MDIFW records, so that observed differences in macroinvertebrate assemblages would be mainly attributed to fish presence. We qualitatively assessed habitat complexity and collected one closed cell pH sample/lake in the field. We compared lake physical conditions (lake surface area, altitude, maximum depth, pH) between fishless and fish-containing lakes with Student's *t*-tests ($\alpha = 0.1$). We verified fish presence and species composition (Table A.1) with the same fish survey methods used in fishless lakes.

3.3.2 Macroinvertebrate sampling and identification

Macroinvertebrates were sampled once per lake during the summers of 2002-2005 with submerged light traps (n = 10/lake) containing glow sticks to attract free-swimming

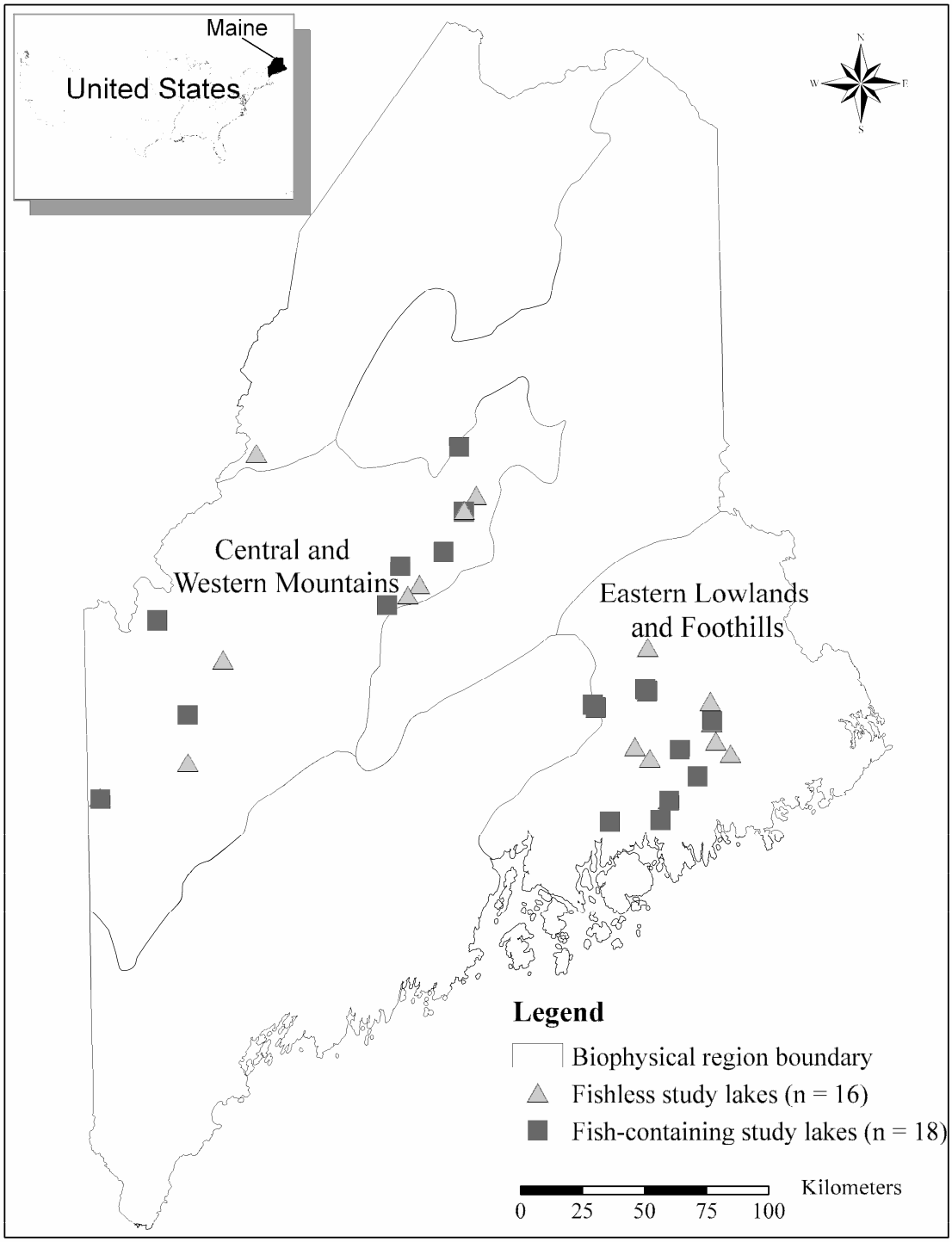

Figure 3.1. Distribution of study lakes in Maine. Biophysical regions are delineated by Krohn *et al.* (1999).

invertebrates (similar to the methods of Newhouse & Stahl, 2000). Submerged light traps were two liter soda bottles with the spout portion cut out and inverted into the remaining bottle. The trap was suspended horizontally in the water column ~0.5m below the water surface by a line attached to a float and anchored with a rock (Figure 3.2). A six-inch, eight-10 hour Cyalume light stick (Omniglow®, Indian Orchard, MA, USA) was activated and placed in each trap before it was deployed. Traps were set by boat in the littoral zone, approximately one meter from the lake edge at dusk and retrieved at dawn (~10 hour deployment). We also collected littoral sweeps (three successive one meter sweeps across the same area) in a subset of 22 lakes (11 fishless, 11 fish-containing) at five sites per lake using a D-net (one mm mesh) to target organisms associated with littoral vegetation. We sieved (1mm mesh) and preserved (70% ethanol) all samples. Each fishless lake was sampled within two days of a fish-containing lake in the same region, and we alternated sampling dates between regions, resulting in no significant differences in sampling date between fishless kettle and headwater lakes or between fishless and fish-containing lakes.

Macroinvertebrates were identified in the laboratory with a dissecting microscope (Table A.2). Coleoptera, Hemiptera, Ephemeroptera, Odonata and *Chaoborus* collected in submerged light traps were counted and identified to genus based on primary literature. Coleoptera, Odonata, Notonectidae and *Chaoborus* were identified to species depending on the life-stage and specimen condition. We limited our analyses of littoral collections to a subset of taxa identified to species, including Coleoptera, Anisoptera and *Notonecta*.

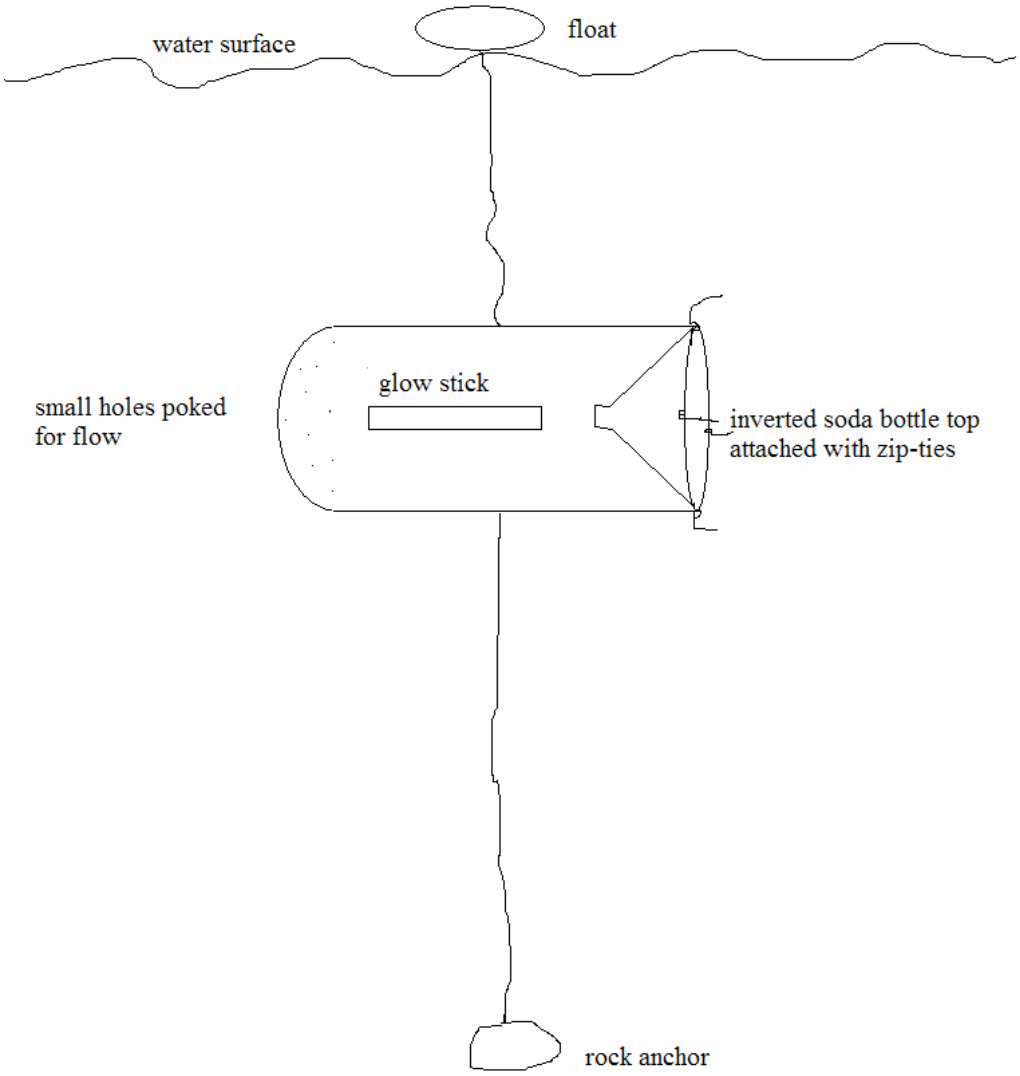

Figure 3.2. Submerged light trap used to attract free-swimming macroinvertebrates.

3.3.3 Statistical analyses

We assessed differences in macroinvertebrate community structure between fishless kettle and headwater lakes with non-metric multidimensional scaling (NMS) for common genera (occurring in > 10% of all study lakes) collected in submerged light traps. This ordination technique uses rank order information in a dissimilarity matrix and is well-suited for community data by avoiding the assumptions of normality and linearity (Clarke, 1993). NMS was run on absolute abundances ($\log_{10}$ x+1 transformed) of common taxa in the autopilot mode ("slow and thorough" thoroughness setting and Sorensen distance measure) in PC-ORD v5 (McCune & Medford, 1999). The optimal dimensionality is found by performing 250 runs on real data followed by 250 runs on randomized data, using random starting configurations for each run and with each run stepping down in dimensionality from six axes to one axis. The best solution selected for each dimensionality is that with the lowest final stress (an inverse measure of fit) from a real run, and the dimensionality is chosen by comparing the final stress values among the best solutions, one best solution for each dimensionality (McCune & Medford, 1999). The final ordination is then obtained using the optimal dimensionality and the starting configuration scores from the best solution. We used autopilot to perform five ordinations and evaluated each for consistency of interpretation. We verified that stress stabilized in each ordination by examining plots of stress vs. iteration, and assessed data structure with Monte Carlo tests (comparing final stress values in randomized vs. real data) and scree plots (stress value vs. the number of dimensions). All five ordinations resulted in the same number of dimensions. We chose the ordination with the lowest final stress value as

the optimum solution. We compared NMS scores between fishless kettle lakes and fishless headwater lakes with Student's *t*-tests ($\alpha = 0.1$).

We compared total absolute macroinvertebrate abundance collected in fishless kettle and headwater lakes, as well as absolute abundances of common (occurring in > 10% of all study lakes) macroinvertebrate taxa, with Student's *t*-tests ($\alpha = 0.1$) to test the hypothesis that macroinvertebrate abundance differs between the two physiographic fishless lake types. Abundances were $\log_{10}$ x+1 transformed prior to analysis. We compared percent occurrence (Fisher's Exact Tests; $\alpha = 0.1$) of common taxa between fishless kettle and headwater lakes to test the hypothesis that macroinvertebrate taxonomic composition differs between the two lake types. We compared species richness of all families that were identified to species and genus richness for Corixidae (Student's *t*-tests; $\alpha = 0.1$) to test the hypothesis that species richness differs between fishless kettle and headwater lakes. Taxon-specific analyses were conducted separately for submerged light trap and littoral sweep collections. We used SYSTAT (version 11, Systat Software Inc., Richmond, CA, USA) for these analyses. We report results at the coarsest level of taxonomic identification showing significant differences. Results for finer taxonomic levels are reported for taxa demonstrating important genus or species associations with either fishless lake type.

We used the same analyses described above to assess differences in macroinvertebrate communities between fishless and fish-containing lakes. For this analysis we pooled data from kettle and headwater lakes, because there were few taxon-specific and community-level differences in fishless lakes between these physiographic lake types. We assessed differences in community structure of fishless and fish-

containing lakes with NMS on absolute abundances ($\log_{10}$ x+1 transformed) of common genera captured in submerged light traps. We compared total absolute and taxon-specific absolute abundances (Student's *t*-tests; $\alpha = 0.1$) and the frequency of occurrence (Pearson Chi-square tests when expected values were > 5, Fisher's Exact Tests when expected values were ≤ 5; $\alpha = 0.1$) of common macroinvertebrate taxa between fishless and fish-containing lakes. We also compared species richness for those families that we identified to species and genus richness for Corixidae (Students *t*-tests; $\alpha = 0.1$). To understand differences in species composition that might be overlooked by common species analysis we calculated percent occurrence of taxa unique to either fishless or fish-containing lakes that were collected in >10% of one lake type and absent in the other lake type. We identified indicators of fish absence as those taxa that were widespread among fishless lakes ($\geq$ 50% lakes) and that were either completely absent from or present in low numbers of fish-containing lakes. Taxon-specific comparisons were made separately for submerged light trap and littoral sweep collections. We compared results between submerged light traps and littoral sweeps to assess whether differences in abundance and percent occurrence were real or a reflection of sampling technique. We also compared capture rates of the two sampling techniques to determine which was more effective at capturing fishless bioindicator taxa. Comparisons between the sample types were made only for the subset of taxa identified from littoral sweeps (Coleoptera, Anistopera, Notonectidae).

3.4 Results

3.4.1 Study lake physical conditions

Fishless kettle and headwater lakes differed significantly in altitude and pH (Table 3.1A). Fishless kettle lakes were low altitude (58 – 140m) and acidic (pH = 4.15 – 5.29); whereas, fishless headwater lakes were high altitude (254 – 893m) and the majority (6/8) had pH > 6.0. Fishless headwater lakes contained scarce aquatic vegetation and habitat structure in the littoral zone. Littoral vegetation structure in fishless kettle lakes was more complex, often with *Sphagnum* mats along the shoreline and lake bottom. Maximum depth and surface area did not differ significantly between the two fishless lake types (Table 3.1A). The majority (7/8) of kettle lakes were seepage lakes inaccessible to fish, and one fishless kettle lake (Mud Pond) was a drainage lake accessible to fish. Paleolimnological analysis of lake sediments found this lake naturally acidic prior to the effects of acid deposition (Davis *et al.*, 1994), and naturally low pH has likely prevented fish survival. Fishless headwater lakes were located in areas of steep terrain where steep outlet streams likely have prevented fish colonization.

Lake pH was the only measured physical variable that differed significantly between fishless and fish-containing study lakes (Table 3.1B), and this difference was attributed to study lakes in the eastern lowlands and foothills ($\mu_{\text{fishless east}} = 4.70$, $\mu_{\text{fish-containing east}} = 5.97$; Student's *t*-test, $t_{[14]} = -5.88$, $P < 0.0001$); pH did not differ significantly between fishless and fish-containing lakes in the central and western mountains ($\mu_{\text{fishless west}} = 6.27$, $\mu_{\text{fish-containing west}} = 6.00$; Student's *t*-test, $t_{[14]} = 0.84$, $P = 0.413$).

Table 3.1. (A) Means and standard errors (SE) of physical characteristics of fishless headwater and kettle lakes. Characteristics are compared using Student's *t*-tests. (B) Same as in (A) but for fishless and fish-containing lakes. Boldface type indicates significant differences (P < 0.1).

A)	*Fishless kettle lakes*		*Fishless headwater lakes*				
	Mean	*SE*	*Mean*	*SE*	*t*	*df*	*p*
Area (ha)	2.57	0.79	2.91	0.65	-0.317	14	0.756
Altitude (m)	84.25	9.15	560.75	75.38	-6.275	7	**0.000**
pH	4.70	0.14	6.27	0.26	-5.377	14	**0.000**
Maximum depth (m)	6.71	1.81	5.87	1.45	0.362	14	0.723
B)	*Fishless lakes*		*Fish-containing lakes*				
	Mean	*SE*	*Mean*	*SE*	*t*	*df*	*p*
Area (ha)	2.75	0.50	3.82	0.50	-1.520	32	0.139
Altitude (m)	322.50	71.62	298.78	66.33	0.243	32	0.809
pH	5.48	0.25	5.98	0.12	-1.827	22	**0.081**
Maximum depth (m)	6.28	1.13	6.60	1.12	-0.199	32	0.843

3.4.2 Comparisons of macroinvertebrate communities in fishless kettle and headwater lakes

Macroinvertebrate communities in fishless kettle lakes and fishless headwater lakes are not distinct based on our NMS analyses of common genera captured in submerged light traps. Three major gradients captured most of the variability in the fishless lakes dataset, with axes 1, 2 and 3 explaining 86.0% of the total variance (r^2 = 0.404, 0.063 and 0.393 respectively). The final stress value (9.355) was low for an ecological community analysis, which generally have stress values ranging 10-20 with values in the lower half of that range considered suitable (McCune & Grace, 2002). Stress is dependent on sample size. This portion of our analysis is based on 16 lakes; therefore, smaller stress values are not unexpected (McCune & Grace, 2002). NMS scores were not significantly different between fishless kettle lakes and fishless headwater lakes on any of the three axes (axis 1: $t_{[14]} = 1.358$, P = 0.196; axis 2: $t_{[14]} = -0.213$, P = 0.834; axis 3: $t_{[14]} = 1.477$, P = 0.162).

A total of 46 Hemiptera, Coleoptera, Odonata and *Chaoborus* taxa were identified from submerged light trap and littoral sweeps, with eight taxa abundant in most fishless lakes (Table 3.2). The total number of captured macroinvertebrates ($\mu_{\text{light trap kettle}} = 2.59$, $\mu_{\text{light trap headwater}} = 2.61$; $\mu_{\text{littoral kettle}} = 1.90$, $\mu_{\text{littoral headwater}} = 1.72$) and total species richness ($\mu_{\text{light trap kettle}} = 9.38$, $\mu_{\text{light trap headwater}} = 8.50$; $\mu_{\text{littoral kettle}} = 5.60$, $\mu_{\text{littoral headwater}} = 5.33$), as well as richness at the family level, did not differ between fishless kettle lakes and fishless headwater lakes. No taxa collected in littoral sweeps showed significant differences in abundance or percent occurrence between fishless kettle lakes and fishless headwater lakes; however, five taxa collected in submerged light traps differed between the two fishless lake types (Figure 3.3). *Buenoa* spp. were more abundant ($t_{[14]} = 2.570$, $P = 0.022$) and occurred more frequently (Fisher's exact $P = 0.026$) in fishless kettle lakes than in fishless headwater lakes. *Tropisternus spp*. were significantly more abundant in fishless kettle lakes ($t_{[7]} = 2.049$, $P = 0.080$). *Hesperocorixa* spp. occurred more frequently in fishless kettle lakes (Fisher's exact $P = 0.077$). *Gyrinus spp*. ($t_{[8]} = -2.465$, $P = 0.039$) and *Neocorixa spp*. ($t_{[8]} = -2.006$, $P = 0.085$) were significantly more abundant in fishless headwater lakes.

Table 3.2. List of common Hemiptera, Coleoptera, Odonata, Ephemeroptera and *Chaoborus* captured in submerged light traps or littoral sweeps in fishless lakes. The most abundant [measured as absolute abundance ("#")] within lakes and widespread [>50% occurrence ("%")] among fishless lakes are indicated. Highlighted cells indicate taxa with significant associations (in terms of abundance and/or percent occurrence) with eastern fishless lakes ("*E*") and with western fishless lakes ("*W*").

Hemiptera
- Belostomatidae
  - *Belostoma spp.*
  - *Lethocerus spp.*
- Nepidae
- Notonectidae
  - *Notonecta insulata # %*
  - *Notonecta undulata*
  - *Buenoa spp. % E*
  - *Buenoa limnocastoris*
  - *Buenoa macrotibialis #*
- Corixidae
  - *Callicorixa spp. %*
  - *Cenocorixa spp.*
  - *Graptocorixa spp.*
  - *Hesperocorixa spp. # % E*
  - *Neocorixa spp. # W*
  - *Sigara spp. # %*

Chaoborus
- *Chaoborus albatus #*
- *Chaoborus americanus # %*
- *Chaoborus punctipennis*
- *Chaoborus trivittatus*

Coleoptera
- Dytiscidae
  - *Desmopachria convexa*
  - *Dytiscus spp.*
  - *Graphoderus liberus # %*
  - *Ilybius discedens*
  - *Laccophilus maculosus*
  - *Matus ovatus*
  - *Thermonectes spp.*
- Gyrinidae
  - *Dineutus spp. # %*
  - *Gyrinus spp. # % W*
- Haliplidae *E*
  - *Haliplus blanchardi %*
  - *Haliplus leopardus*
  - *Haliplus longulus*
  - *Peltodytes spp.*
- Hydrophilidae
  - *Tropisternus spp. E*

Odonata
- Aeshnidae
  - *Aeshna eremita %*
  - *Aeshna interrupta*
- Coenagrionidae
  - *Enallagma spp. %*
- Lestidae
  - *Lestes rectangularis*
  - *Lestes unguiculatus*
- Libellulidae
  - *Ladona julia*
  - *Leucorrhinia glacialis # %*
  - *Leucorrhinia frigida*
  - *Leucorrhinia hudsonica*
  - *Leucorrhinia patricia #*
- Corduliidae
  - *Cordulia shurtleffi %*

Ephemeroptera
- Ephemerellidae
  - *Eurylophella spp.*
- Leptophlebidae
- Siphlonuridae

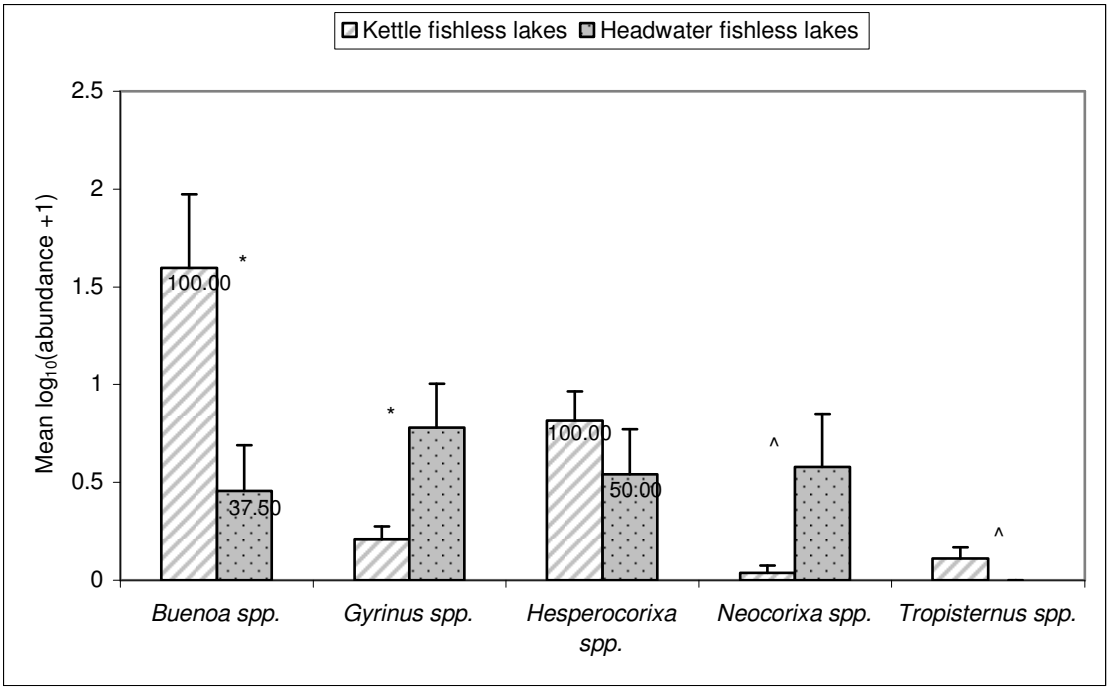

Figure 3.3. Mean abundance of macroinvertebrates showing significant associations with fishless kettle or headwater lakes. Significant differences are indicated by ^ ($P<0.1$) and * ($P<0.05$). Abundance is total number of individuals captured in 10 submerged light traps. Bars indicate means +1 SE. Percent occurrence is noted inside bars for taxa demonstrating significant differences ($P<0.1$).

3.4.3 Comparisons of macroinvertebrate communities in fishless and fish-containing lakes

Taxa associated with fishless lakes: Fishless and fish-containing lakes support distinct macroinvertebrate communities based on our NMS analysis of common genera captured in submerged light traps. Three major gradients captured most of the variability in the dataset, with axes 1, 2 and 3 explaining 82.4% of the total variance ($r^2 = 0.265$, 0.208 and 0.351, respectively). The final stress value was 14.44. NMS scores on axis 1 were not significantly different between fishless and fish-containing lakes ($t_{[23]} = -1.196$, $P = 0.244$); however, differences in scores were significant on axis 2 ($t_{[32]} = 3.292$, $P = 0.002$) and axis 3 ($t_{[20]} = 6.085$, $P < 0.0001$; Figure 3.4). Fishless lakes tended to score

negatively, and fish-containing lakes tended to score positively on axes 2 and 3 (Figure 3.4). Genera negatively correlated with axes 2 and 3, indicating significant associations with fishless lakes, were *Buenoa* spp., *Notonecta* spp., *Callicorixa* spp., *Hesperocorixa* spp., *Sigara* spp., *Dineutus* spp., *Gyrinus* spp., *Graphoderus* spp. and *Ilybius* spp. (Table 3.3). *Cenocorixa* spp. was the only genus positively correlated with axis 3, indicating association with fish-containing lakes (Table 3.3).

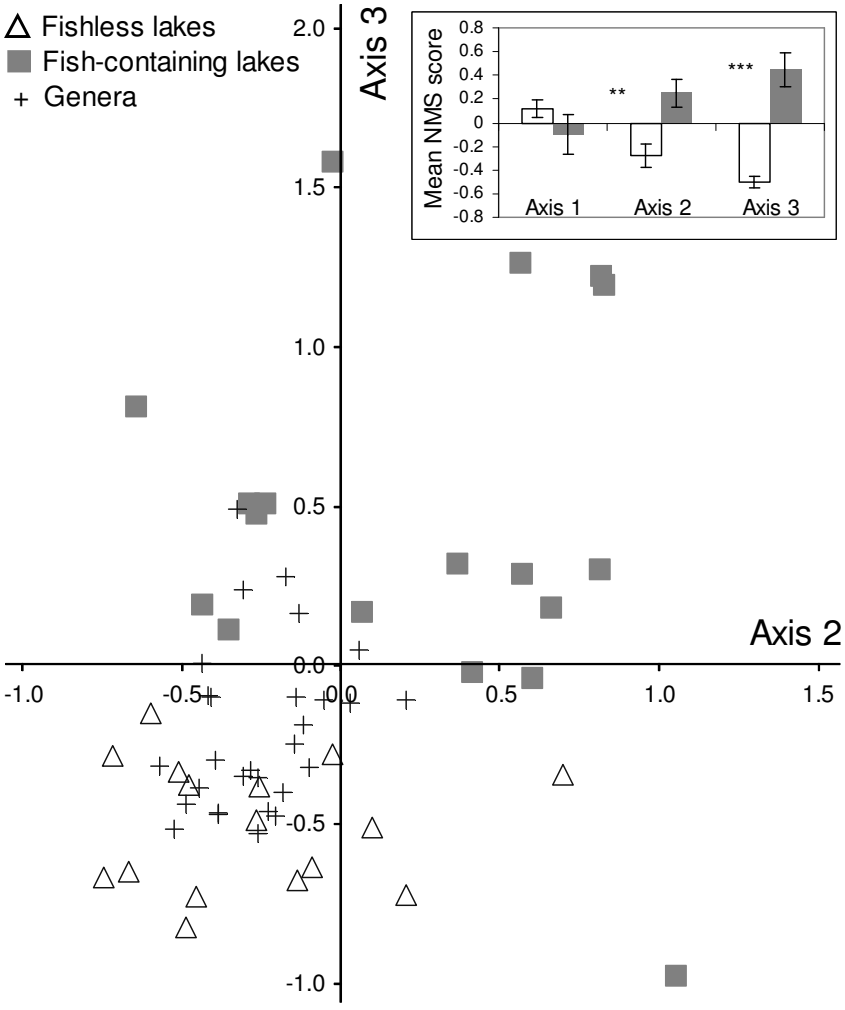

Figure 3.4. NMS scores on axes 2 and 3 for lakes and genera. Inset figure shows mean NMS scores for fishless and fish-containing lakes (±1 SE). Significant differences are indicated by ** (P<0.01) and *** (P<0.001).

Table 3.3. Pearson correlation coefficients of macroinvertebrate genera with NMS axes for community level analysis of fishless and fish-containing lakes. Boldface type indicates significant r values ($\alpha = 0.1$). Negative correlations with axes 2 and 3 indicate significant associations with fishless lakes; positive correlations with axes 2 and 3 indicate significant associations with fish-containing lakes.

Family	*Genus*	*Axis 1*	*Axis 2*	*Axis 3*
Notonectidae	*Buenoa*	**0.35**	**-0.38**	**-0.36**
	Notonecta	0.11	**-0.58**	**-0.40**
Corixidae	*Callicorixa*	-0.01	**-0.49**	**-0.39**
	Cenocorixa	0.20	-0.26	**0.33**
	Graptocorixa	0.25	**-0.29**	-0.06
	Hesperocorixa	0.11	**-0.36**	**-0.58**
	Neocorixa	-0.13	-0.16	**-0.30**
	Palmacorixa	-0.05	-0.19	0.11
	Sigara	-0.12	**-0.70**	**-0.43**
Belosomatidae	*Lethocerus*	0.13	**-0.43**	-0.08
Nepidae	*Ranatra*	0.02	-0.09	-0.05
Gyrinidae	*Dineutus*	0.07	**-0.60**	**-0.44**
	Gyrinus	0.11	**-0.37**	**-0.34**
Dystiscidae	*Dytiscus*	0.07	**-0.53**	-0.24
	Graphoderus	0.20	**-0.62**	**-0.61**
	Ilybius	0.06	**-0.31**	**-0.30**
	Laccophilus	-0.05	**-0.34**	0.00
	Matus	-0.21	-0.20	**-0.33**
	Thermonectes	0.26	-0.08	-0.10
Haliplidae	*Haliplus*	0.03	0.11	0.08
	Peltodytes	**-0.37**	-0.09	-0.23
Hydrophilidae	*Tropisternus*	0.17	-0.12	-0.16
Aeshnidae	*Aeshna*	0.25	-0.23	**-0.40**
Libellulidae	*Leucorrhinia*	-0.20	-0.26	-0.28
Coenagrionidae	*Enallagma*	**0.35**	0.24	-0.11
Lestidae	*Lestes*	**0.31**	0.03	-0.10
Chaoboridae	*Chaoborus*	0.10	-0.13	-0.22
Ameletidae	*Ameletus*	-0.06	-0.23	0.27
Siphloniuridae	*Siphlonurus*	0.19	-0.07	0.07

Results from taxon-specific analyses of submerged light traps in fishless and fish-containing lakes supported the community-level NMS results. The total number of macroinvertebrates captured in submerged light traps was greater in fishless lakes (μ = 2.60) than fish-containing lakes (μ = 1.75; $t_{[32]} = -3.819$, $P < 0.001$), with greater abundances of Hemiptera, Coleoptera and Odonata in fishless lakes (Figure 3.5; Table 3.4). Hemipterans associated with fishless lakes were Notonectidae and Corixidae (Figure 3.6; Table 3.4), with *Buenoa* spp., *Notonecta insulata* (Kirby), *Callicorixa* spp., *Hesperocorixa* spp. and *Sigara* spp. more abundant and present in more fishless lakes (Figure 3.7; Table 3.4). Coleopterans associated with fishless lakes were Gyrinidae and Dytiscidae (Figure 3.6; Table 3.4), with *Dineutus* spp., *Gyrinus* spp., *Graphoderus liberus* (Say), *Ilybius* spp. and *Thermonectes* spp. more abundant and present in more fishless lakes (Figure 3.7; Table 3.4). Odonates associated with fishless lakes were Aeshnidae, Libellulidae and Coenagrionidae (Figure 3.6; Table 3.4), with *Aeshna eremita* (Scudder) and *Leucorrhinia glacialis* (Hagen) more abundant, as well as present in more lakes lacking fish (Figure 3.7; Table 3.4). *Chaoborus americanus* (Johannsen) also was both more abundant, and more ubiquitous, in fishless lakes (Figure 3.7; Table 3.4).

Mean richness of all species identified in submerged light traps was more than two times greater in fishless lakes than in lakes containing fish ($\mu = 8.9_{\text{fishless}}$, $\mu_{\text{fish-containing}} = 4.0$; $t_{[32]} = -0.420$, $P = 0.0001$), with six of ten families more speciose in fishless lakes: Dytiscidae ($t_{[17]} = -5.401$, $P < 0.0001$), Gyrinidae ($t_{[16]} = -2.079$, $P = 0.054$), Chaoboridae ($t_{[32]} = -2.014$, $P = 0.052$), Notonectidae ($t_{[23]} = -3.026$, $P = 0.006$), Aeshnidae ($t_{[32]} = -2.189$, $P = 0.036$) and Libellulidae ($t_{[18]} = -1.832$, $P = 0.083$; Figure 3.8). Corixidae demonstrated the highest richness at the genus level in our collections, with eight genera

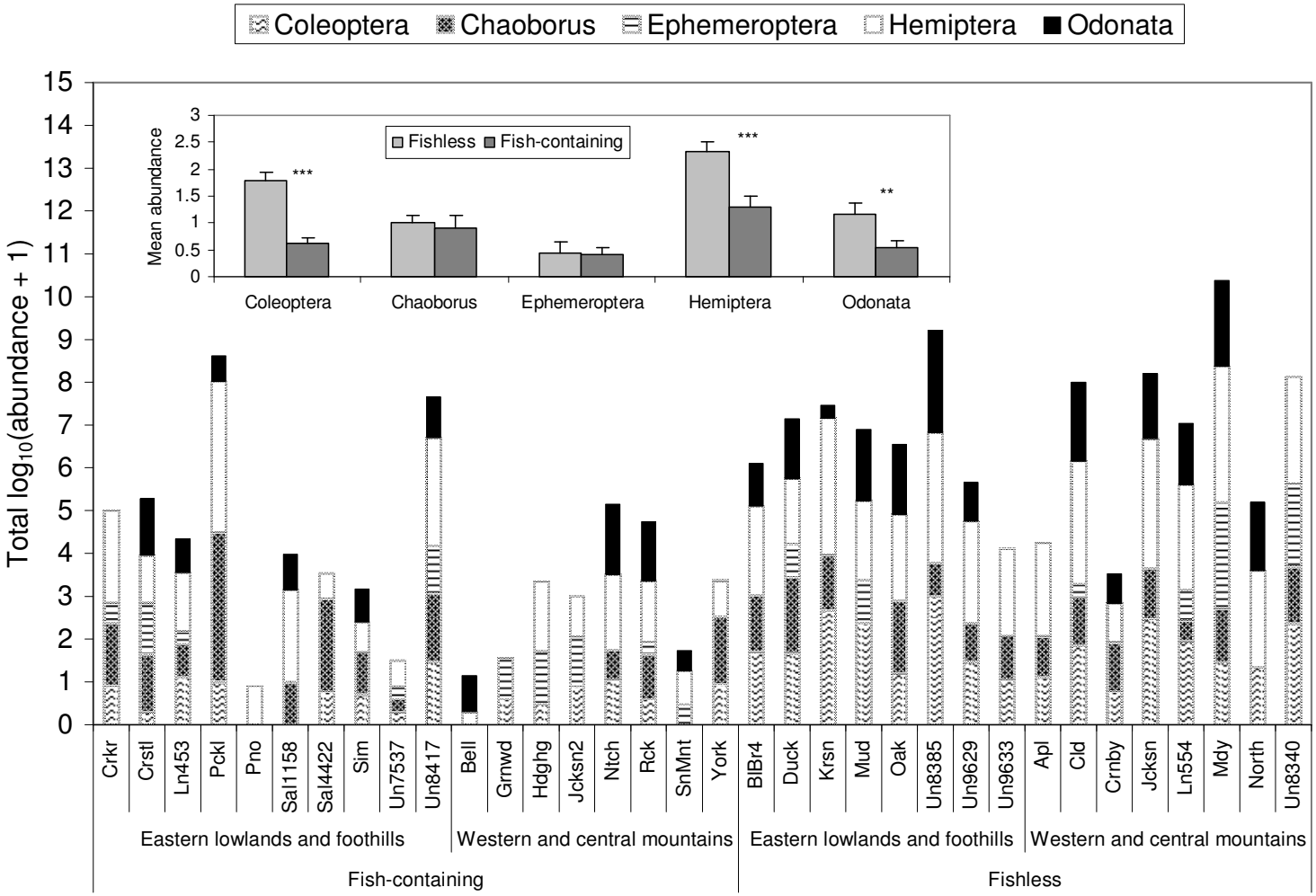

Figure 3.5. Total abundance of macroinvertebrates in submerged light traps (10/lake). Lake names are abbreviated. Inset figure shows average $\log_{10}(x+1)$ transformed abundance by lake type (n = 16 fishless, n =18 fish-containing). Significant differences are indicated by ** ($P<0.01$) and *** ($P<0.001$).

Table 3.4. Mean abundance (standard error) and percent occurrence of common taxa showing differing distributional patterns between fishless and fish-containing lakes. Data are presented at the most aggregated taxonomic level with significant differences. Finer taxonomic levels also are presented for taxa with genus or species associations with either lake type. Test statistics and p-values compare [$\log_{10}(x+1)$ transformed] abundance (Student's *t*-tests) and percent occurrence [Pearson Chi-square (when expected values were >5) and Fisher's Exact Tests (when expected values were ≤ 5)] between fishless and fish-containing lakes. Highlighted taxa indicate significant associations with fishless (light grey) and fish-containing (dark grey) lakes. Data are for taxa collected in submerged light traps except for *Leucorrhinia glacialis,* which was common only in littoral collections. No test statistic is generated with Fisher's Exact Tests; df = 1 for all 2x2 contingency tables.

	Abundance					*Percent occurrence*			
	Fishless	*Fish-containing*	*t stat*	*df*	*p*	*Fishless*	*Fish-containing*	*Pearson χ2*	*p*
Hemiptera	2.34 (0.16)	1.29 (0.21)	-3.952	32	0.000	100.00	94.44	na	1.000
Notonectidae	1.74 (0.25)	0.30 (0.09)	-5.42	19	0.000	93.75	50.00	na	0.008
Buenoa spp.	1.03 (0.26)	0.20 (0.08)	-3.038	18	0.007	68.75	33.33	4.25	0.039
Notonecta insulata	0.52 (0.15)	0.00 (0.00)	-3.56	15	0.003	56.25	0.00	na	0.000
Corixidae	1.95 (0.15)	1.22 (0.21)	-2.73	32	0.010	100.00	94.44	na	1.000
Callicorixa spp.	0.35 (0.11)	0.00 (0.00)	-3.25	15	0.005	50.00	0.00	na	0.001
Cenocorixa spp.	0.24 (0.09)	0.70 (0.18)	2.35	25	0.027	37.50	83.33	7.54	0.006
Hesperocorixa spp.	0.68 (0.14)	0.05 (0.04)	-4.39	17	0.000	75.00	11.11	14.28	0.000
Sigara spp.	0.87 (0.16)	0.30 (0.11)	-2.97	32	0.006	75.00	38.89	4.48	0.034
Gerridae	0.00 (0.00)	0.09 (0.04)	2.13	17	0.048	0.00	22.22	na	0.105
Coleoptera	1.78 (0.16)	0.62 (0.11)	-6.17	32	0.000	100.00	77.78	na	0.105
Gyrinidae	0.95 (0.16)	0.16 (0.08)	-4.33	22	0.000	87.50	27.77	12.26	0.000
Dineutus spp.	0.69 (0.17)	0.09 (0.07)	-3.33	21	0.003	68.75	11.11	11.92	0.001
Gyrinus spp.	0.50 (0.13)	0.07 (0.04)	-3.07	18	0.007	68.75	16.67	9.49	0.002
Dytiscidae	1.60 (0.19)	0.20 (0.06)	-7.06	18	0.000	93.75	44.44	9.41	0.002
Graphoderus liberus	1.09 (0.15)	0.00 (0.00)	-7.08	15	0.000	93.75	0.00	30.20	0.000
Ilybius spp.	0.16 (0.06)	0.00 (0.00)	2.13	15	0.019	37.50	0.00	na	0.006
Thermonectes spp.	0.15 (0.07)	0.00 (0.00)	2.04	32	0.036	25.00	0.00	na	0.039
Odonata	1.17 (0.20)	0.53 (0.13)	-2.71	32	0.011	81.25	55.56	2.56	0.110
Aeshnidae	0.44 (0.12)	0.11 (0.05)	-2.549	21	0.019	62.50	22.22	5.67	0.017
Aeshna eremita	0.26 (0.09)	0.04 (0.03)	-2.18	18	0.043	43.75	11.11	na	0.052
Libellulidae									
*Leucorrhinia glacialis**	1.20 (0.24)	0.27 (0.10)	-3.45	14	0.004	45.46	22.73	na	0.063
Coenagrionidae	0.83 (0.20)	0.33 (0.10)	-2.22	23	0.037	68.75	44.44	2.03	0.154
Diptera									
Chaoboridae									
Chaoborus americanus	0.54 (0.13)	0.00 (0.00)	-4.23	15	0.000	62.50	0.00	15.94	0.000
Chaoborus punctipennis	0.21 (0.10)	0.82 (0.23)	2.45	24	0.022	25.00	55.56	3.27	0.071

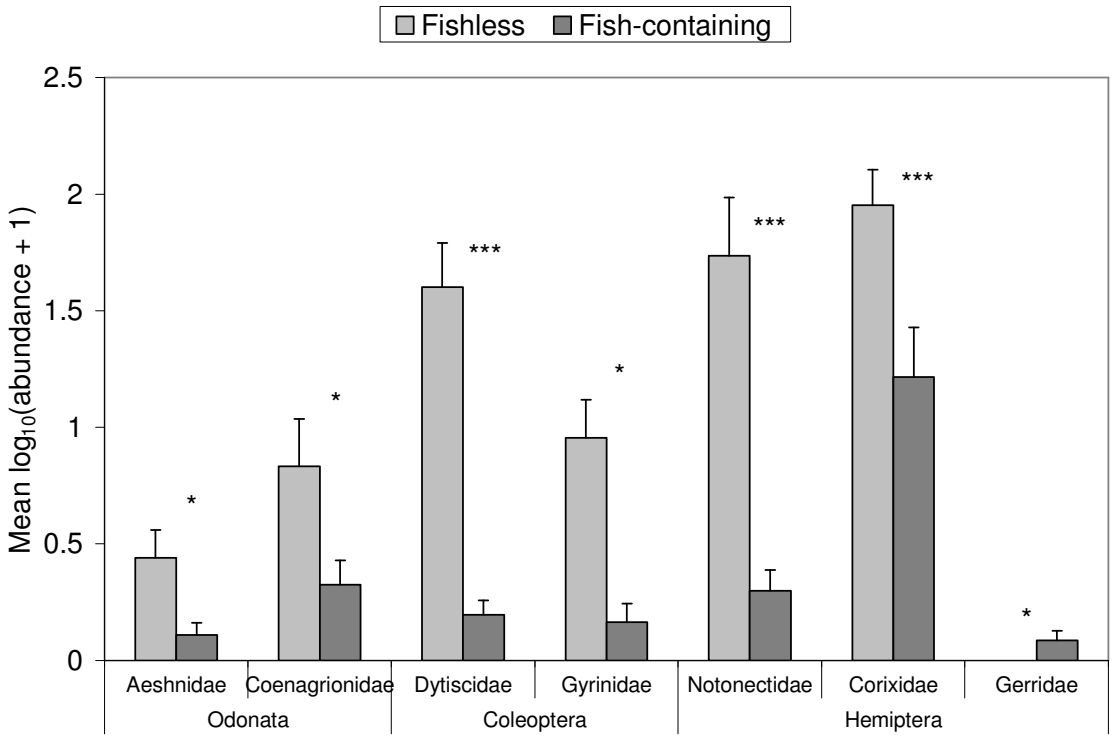

Figure 3.6. Mean abundance of families collected in light traps showing significant associations with fishless or fish-containing lakes. Significant differences are indicated by * ($P<0.05$) and *** ($P<0.001$). Abundance is total number of individuals captured in 10 submerged light traps. Bars indicate mean +1 SE.

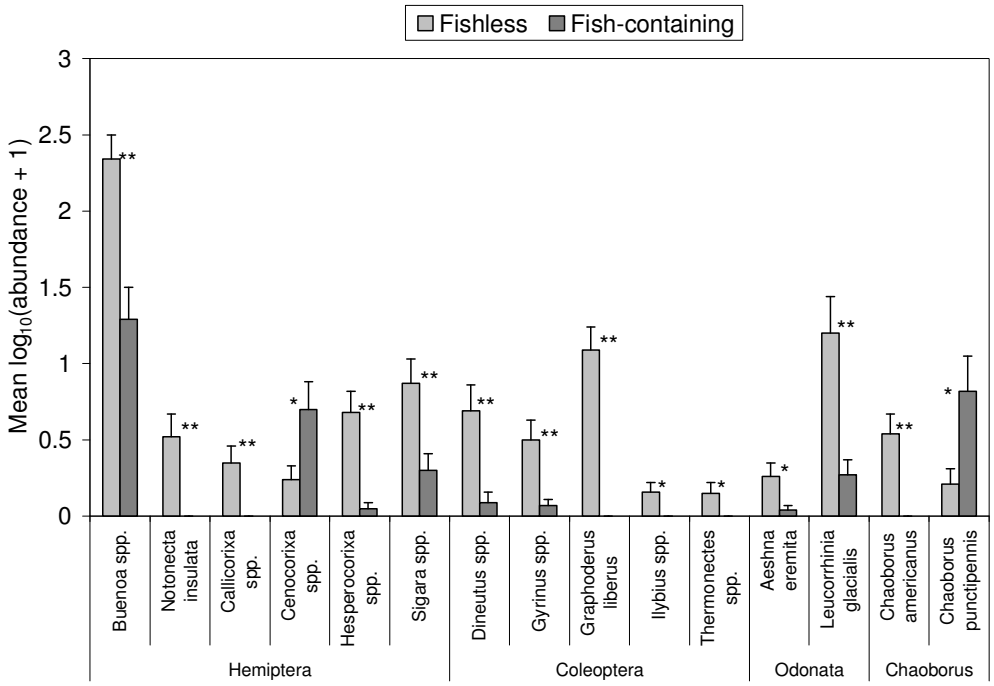

Figure 3.7. Mean abundance of genera and species collected in light traps showing significant associations with fishless or fish-containing lakes. Significant differences are indicated by * (P<0.05), ** (P<0.01) and *** (P<0.001). Abundance is total number of individuals captured in 10 submerged light traps. Bars indicate means +1 SE. *Leucorrhinia glacialis* data are from littoral collections.

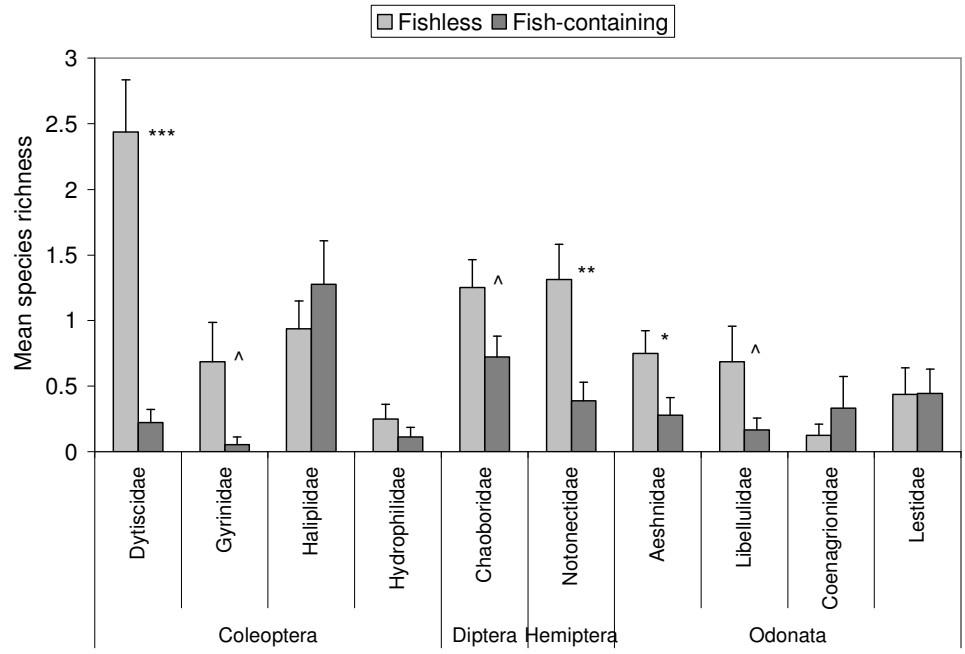

Figure 3.8. Mean species richness for families collected in light traps identified to species in fishless and fish-containing lakes. Bars indicate means +1 SE. Significant differences are indicated by ^ (P<0.1), * (P<0.05), ** (P<0.01) and *** (P<0.001).

collected (*Arctocorixa, Callicorixa, Cenocorixa, Graptocorixa, Hesperocorixa, Neocorixa, Palmacorixa, Sigara*). Fishless lakes contained significantly more genera of Corixidae than fish-containing lakes ($\mu_{fishless} = 2.938$, $\mu_{fish\text{-}containing} = 1.889$; $t_{[32]} = -2.083$, $P = 0.045$).

Twelve taxa collected in submerged light traps were restricted to fishless lakes. Seven species were unique but not widespread among fishless lakes, including the chaoborid *Chaoborus trivittatus* (Loew) in two lakes, the dytiscid *Desmopachria convexa* (Aubé) in three lakes, four gyrinids [*Dineutus ciliatus* (Forsberg) in two lakes, *Dineutus emarginatus* (Say) in two lakes, *Dineutus nigrior* (Roberts) in three lakes, *Gyrinus dubius* (Wallis) in four lakes] and the libellulid *Leucorrhinia patricia* (Walker) in two lakes. Four taxa (*Callicorixa* spp., *Chaoborus americanus*, *Notonecta insulata*, *Graphoderus liberus*) were unique to and widespread among fishless lakes (Figure 3.7; Table 3.4). We identified as bioindicators of fish absence, these four taxa plus *Hesperocorixa* spp. and *Dineutus* spp., as these six taxa were widespread among fishless lakes and found in only two fish-containing lakes (Table 3.5). *Graphoderus liberus* was the most widespread bioindicator, occurring in all but one fishless lake (Table 3.5). Fifteen of 16 fishless lakes supported at least three of the six indicator taxa, with *Hesperocorixa* spp., *Dineutus spp,*. and *Graphoderus* spp. co-occurring most frequently (eight/16 lakes; Table 3.5).

Table 3.5. Occurrence of fishless indicator taxa (+ indicates present, - indicates absent) in fishless lakes. Indicators were widespread among fishless lakes (≥ 50% lakes) and were either completely absent from or occurred rarely in fish-containing lakes.

Lake name	*Lake type*	Graphoderus liberus	Hesperocorixa spp.	Dineutus spp.	Chaoborus americanus	Notonecta insulata	Callicorixa spp.
Apple Pd	headwater	+	+	-	+	-	+
Cloud Pd	headwater	+	-	+	+	+	+
Cranberry Pd	headwater	-	-	-	+	+	-
Jackson Pd	headwater	+	-	+	+	+	+
Loon Pd 554	headwater	+	+	+	-	-	-
Midday Pd	headwater	+	+	-	-	+	+
North Pd	headwater	+	+	+	-	-	+
Unnamed 8340	headwater	+	-	+	+	+	-
Black Brook #4	kettle	+	+	-	+	+	-
Duck Pd	kettle	+	+	+	-	+	-
Kerosene Pd	kettle	+	+	+	+	-	+
Mud Pd 4420	kettle	+	+	+	-	+	+
Oak Pd	kettle	+	+	+	+	-	-
Unnamed 8385	kettle	+	+	+	-	-	+
Unnamed 9629	kettle	+	+	-	+	-	-
Unnamed 9633	kettle	+	+	+	+	+	-
% of fishless lakes where taxa is present		93.75	75.00	68.75	62.50	56.25	50.00
% fish-containing lakes where taxa is present		0.00	11.11	11.11	0.00	0.00	0.00

Taxa associated with fish-containing lakes: Gerridae was the only common taxon restricted to fish-containing lakes (Figure 3.6; Table 3.4). *Cenocorixa* spp. and *Chaoborus punctipennis* (Say) were more abundant and present in more fish-containing lakes than fishless lakes (Figure 3.7; Table 3.4). No families were significantly more speciose in fish-containing lakes. Four species were unique to fish-containing lakes, including two coenagrionids, *Enallagma geminatum* (Kellicot) and *Enallagma carunculatum* (Morse), the lestid *Lestes vigilax* (Hagen) and the haliplid *Haliplus connexus* (Matheson). None of these was widespread, each occurring in two fish-containing lakes.

Comparisons of littoral sweep collections and light trap collections: Similar to light traps, total abundance for taxa (Coleoptera, Hemiptera, Anisoptera) identified in littoral sweeps was significantly greater in fishless lakes ($\mu_{fishless} = 1.80$, $\mu_{fish\text{-}containing} =$

1.25; $t_{[20]} = -3.032$, $P = 0.007$). At the taxon-specific level, patterns in abundance and percent occurrence in fishless and fish-containing lakes in littoral sweeps reflected those found in submerged light traps (Table A.3), however, there were differences in capture rates between the two sampling methods. For example, fewer Coleoptera ($\mu_{littoral} = 0.44$, $\mu_{light\ trap} = 1.22$, $t_{[42]} = -3.325$, $P = 0.002$) and Notonectidae ($\mu_{littoral} = 0.53$, $\mu_{light\ trap} = 1.07$, $t_{[42]} = -1.932$, $P = 0.059$) were captured in littoral sweeps than submerged light traps; whereas, littoral sweeps captured more anisopterans than submerged light traps ($\mu_{littoral} = 1.33$, $\mu_{light\ trap} = 0.44$, $t_{[42]} = 5.29$, $P < 0.0001$). Comparing results from littoral sweeps and submerged light traps for individual taxa, significant differences between fishless and fish-containing lakes for some taxa were found only in the sampling method with a higher capture rate (Table A.3). Three taxa identified from submerged light trap collections as fishless bioindicators belonged to taxonomic groups similarly identified in littoral sweeps: *Graphoderus liberus, Dineutus* spp. and *Notonecta insulata*. Similar to light traps, these taxa were not captured in littoral sweeps in fish-containing lakes. Capture rates for all three of these taxa were greater in submerged light traps than littoral sweeps, with *Dineutus* spp. and *Notonecta insulata* not commonly captured in littoral sweeps (Table A.3).

3.5 Discussion

We found few differences between macroinvertebrate communities in fishless kettle lakes in the eastern lowlands and foothills and fishless headwater lakes in the central and western mountains; whereas, similar comparisons of fishless and fish-containing lake communities revealed numerous differences in macroinvertebrate community structure, abundance, taxonomic composition and species richness. Fish

presence or absence was a stronger determinant of community structure in our study lakes than differences in lake origin and physiography. This is consistent with previous studies of fishless and fish-containing lakes, which have found that the distribution and abundance of many aquatic insects is driven primarily by the occurrence of fish predators rather than differences in environmental variables, such as pH (Eriksson *et al.*, 1980; Bendell, 1986; Bendell & McNicol, 1987; McNicol *et al.,* 1987; Brett, 1989; McPeek, 1990*a*; Arnott & Jackson, 2006) and habitat structure (Bennett & Streams, 1986; Binckley & Resetartiz, 2005). Our results indicate that previously reported differences in macroinvertebrate communities between fish-containing and fishless lakes reported in acidified lakes in eastern North America (e.g. Bendell, 1986; Bendell & McNicol, 1987; McNicol *et al.*, 1987) also occur between fish-containing and naturally fishless lakes that are broadly distributed across the landscape. This strong fish effect indicates that widespread fish introductions into naturally fishless lakes may lead to regional declines in native aquatic biodiversity and of this unique aquatic system.

3.5.1 Macroinvertebrate communities in fishless kettle and headwater lakes

We expected macroinvertebrate communities to be distinct between fishless kettle and headwater lakes, given their contrasting physical conditions and landscapes; however, only five taxa were associated with a particular lake type. Macroinvertebrate taxonomic richness and total abundance has been shown to decrease with pH (Friday, 1987; Mallory *et al.*, 1994; McNicol *et al.*, 1995) and some taxa are acid-sensitive [e.g. Ephemeroptera (Bell, 1971; Friday, 1987; Carbone *et al.*, 1998; Snucins, 2003)]. The influence of littoral habitat structure on macroinvertebrate abundance and community structure has been widely demonstrated (Gerking, 1962; Gerrish & Bristow, 1979;

Schmude *et al.*, 1998; Butler & deMaynadier, 2008). Abundances of acid tolerant macroinvertebrates have been shown to be greater in *Sphagnum*, which provides refuge and foraging sites (Henrikson, 1993). While habitat structure is normally thought to benefit macroinvertebrates by providing refuge from fish predation (Crowder & Cooper, 1982; Cook & Streams, 1984; Gilinsky, 1984), studies have shown that macroinvertebrate biomass, species richness and density are positively related to vegetation structure in fishless environments (Gilinsky, 1984; Diehl, 1992).

We expected fishless kettle lakes, which were acidic and had prolific littoral and benthic *Sphagnum* mats, to support greater numbers of acid-tolerant taxa than fishless headwater lakes, which were less acidic and had little habitat structure. Only three acid-tolerant taxa [*Buenoa* spp.*, Tropisternus* spp.*, Hesperocorixa* spp. (Griffiths, 1973; Bendell, 1986)] were associated with fishless kettle lakes and not with fishless headwater lakes. Otherwise, acid-tolerant taxa [Coleoptera (Foster, 1995; Arnott & Jackson, 2006), *Chaoborus* (Yan *et al.*, 1985), Notonectidae (Bendell & McNicol, 1987), Corixidae (Bendell & McNicol, 1987; Werner & McPeek, 1994; Longcore *et al.*, 2006), Odonata (Bell, 1971; Hudson & Berrill, 1986), *Aeshna* (Bendell & McNicol, 1995), *Leucorrhinia glacialis* (Bendell & McNicol, 1995), *Cordulia shurtleffi* (Bendell & McNicol, 1995)] were widespread and abundant in both fishless lake types, and two acid-tolerant taxa (*Gyrinus* spp.*, Neocorixa* spp.) were associated with fishless headwater lakes.

3.5.2 Macroinvertebrate communities in fishless and fish-containing lakes

Taxa associated with fishless lakes – Two families of Hemiptera [Notonectidae (Bendell, 1986; Bendell & McNicol, 1987; Brett, 1989) and Corixidae (Henrikson & Oscarson, 1978; Bendell & McNicol, 1987; Brett, 1989)] tend to thrive in fishless

environments relative to those containing fish. This is particularly evident for the notonectids *Buenoa* spp. (Hurlbert & Mulla, 1981; Brett, 1989) and *Notonecta* spp. (Macan, 1976; Hurlbert & Mulla, 1981; Cook & Streams, 1984; Bennett & Streams, 1986; Brett, 1989) and the corixids *Hesperocorixa* spp. (Macan, 1976; Bendell & McNicol, 1987; Brett, 1989; Bradford *et al.*, 1998), *Callicorixa* spp. (Collinson *et al.*, 1995) and *Sigara* (Macan, 1976; Bendell & McNicol, 1987; Oscarson, 1987; Brett, 1989; Bradford *et al.*, 1998). Although habitat characteristics [e.g. water depth and cover (Taylor, 1968; Streams & Shubeck, 1982; Bennett & Streams, 1986)] and invertebrate predation [especially cannibalism (Sih, 1982)] influence notonectid and corixid species distributions among lakes, fish presence or absence is thought to be the primary factor explaining observed species distributions among lakes. Populations of Notonectidae and Corixidae are reduced substantially after the introduction of fish into previously fishless waters (Macan, 1965; Weir, 1972) due to their vulnerability to fish predation. These taxa are relatively large and must periodically swim to the water surface to breathe, making them visible and frequently exposed to fish predators.

Our results are consistent with other studies in eastern North America demonstrating a strong effect of fish presence on beetle assemblage structure, particularly Dytiscidae (Fairchild *et al.*, 2000). Others have also found that Dytiscidae abundance and distribution are affected more by fish presence than environmental variables such as pH (Bendell & McNicol, 1987; McNicol *et al.*, 1995; Arnott & Jackson, 2006), water depth (Arnott & Jackson, 2006) and lake surface area (Arnott & Jackson, 2006).

The positive association of Gyrinidae (especially *Dineutus* spp. and *Gyrinus* spp.) with fishless lakes was unexpected. Although conspicuous surface-dwellers, these beetles

are relatively immune to predation (Benfield, 1972; Stenson, 1979), because most secrete volatile compounds that repel vertebrate predators (Miller *et al.*, 1975; Miller & Mumma, 1976*a,b*; Scrimshaw & Kerfoot, 1987). Species' ability to produce these compounds has been linked to their habitat use and behavior (Borg Karlsson *et al.*, 1999). Chemical producing species tend to aggregate in rafts on the water surface in open water habitats (Borg Karlsson *et al.*, 1999), which may concentrate their toxins and increase their recognition by predators (Benfield, 1972; Heinrich & Vogt, 1980; Vulinec & Miller, 1989). Species lacking the compounds form looser aggregations, are more solitary, spend more time below the water surface (Fitzgerald, 1987; Borg Karlsson *et al.*, 1999), and often are the only gyrinid species found in small fishless waterbodies (Borg Karlsson *et al.*, 1999). One gyrinid (*Dineutus nigrior*) we found restricted to fishless lakes produces defensive chemicals (Miller *et al.*, 1975), although its behavior is similar to other non-chemical producing gyrinids living primarily in fishless habitats (Fitzgerald, 1987). The large number of gyrinids in Maine's fishless lakes may be species that are more vulnerable to fish predation either due to lack of chemical defenses or to behaviors that make them more vulnerable to fish predation (see section on fishless bioindicators for more detail).

Odonate abundance and species distributions are strongly related to fish presence or absence (Morin, 1984; McPeek, 1990*a*; Carbone *et al.*, 1998; McPeek, 1998; Stoks & McPeek, 2003). Species associated with fishless habitats tend to be large, active, visual predators compared with the small, slow-moving, cryptic, tactile feeders that coexist with fish (Johnson & Crowley, 1980; Blois-Heulin *et al.*, 1990; Bendell & McNicol, 1995; Johansson *et al.*, 2006). Maine's fishless lakes supported greater abundance and percent

occurrence of Aeshnidae, a family that includes the largest dragonfly species in North America, with *Aeshna eremita* showing a positive association with fishless study lakes. Other studies also have found that *Aeshna* spp. tend to be more abundant in fishless lakes (Johnson & Crowley, 1980; Knapp *et al.*, 2005). The libellulid *Leucorrhinia glacialis* was the most abundant dragonfly in our fishless lakes, similar to fishless lakes elsewhere in the Northeast (Bendell & McNicol, 1995; Strong & Robinson, 2004). *Leucorrhinia* spp. dominates odonate assemblages in fishless lakes, suggesting that it may be one of the top predators in these lakes (Bendell & McNicol, 1995; Strong & Robinson, 2004; this study). *Leucorrhinia* spp. are vulnerable to fish predation compared with other libellulids that coexist with fish, because they actively forage during light periods and do not often hide in benthic debris (Nilsson, 1981; Johansson, 1991). Their predator escape behavior (i.e., attempting escape when attacked by fish) may be less successful than other dragonfly genera that are able to coexist with fish by feigning death when attacked (Henrikson, 1988).

Taxa associated with fish-containing lakes – The hemipteran family Gerridae was the only taxon common in fish-containing lakes and absent from fishless lakes in our study. Similarly, in an acidified region of eastern Ontario, Gerridae was absent from fishless lakes, and two species were collected in fish-containing lakes (Bendell & McNicol, 1987). The pattern did not hold in non-acidified lakes, however, where one species was abundant in both fishless and fish-containing lakes. Bendell & McNicol (1987) suggest that these bugs may be acid-sensitive, and their absence from acidified lakes may result from low pH rather than fish absence. Given that Gerridae was absent

from all of our fishless study lakes, pH does not seem to influence their distribution in Maine.

Chaoborus punctipennis occurred more frequently and was more abundant in fish-containing lakes. Positive associations between fish presence and *C. punctipennis* presence and abundance have been well-documented (e.g. Yan *et al.*, 1985; Wissel *et al.*, 2003). Like most chaoborids, this species undergoes diel vertical migration in the presence of fish. *Chaoborus punctipennis* is not strongly affected by fish predation and is able to coexist with fish, because it is small, transparent, and retreats to deep water or sediments during the day. Conversely, it is vulnerable to larger chaoborids through predation and competition for zooplankton prey (Roth, 1968; von Ende, 1979; von Ende, 1982). This species may be more affected by the presence of larger *Chaoborus* species than by fish (Wissel & Benndorf, 1998). Abundance of the larger *C. americanus* in Maine's fishless lakes may explain the near absence of *C. punctipennis* in these lakes.

3.5.3 Bioindicators of the fishless condition

Most taxa we identified as bioindicators of fishless lakes demonstrate extremes of characteristics that increase invertebrate vulnerability to fish predation (e.g. large-bodied, active, conspicuous). The bioindicators discussed below represent a suite of taxa that together indicate "fishlessness." Fifteen of 16 fishless lakes surveyed during this study supported at least three of six taxa identified as fishless bioindicators, suggesting that the presence of three or more is a reliable indication of fish absence.

Graphoderus liberus emerged as the most common indicator of fish absence (Table 3.5). The distribution of this large dytiscid previously has been shown to be strongly negatively associated with fish presence (Bendell & McNicol, 1987; Brett, 1989;

Arnott & Jackson, 2006). Unlike those of most other dytiscid beetles, the larvae of this species are pelagic, making them highly susceptible to fish predation (Larson, 1990). *Dineutus* spp., another coleopteran identified as a fishless indicator, was found in a majority of fishless lakes and in only two fish-containing lakes. Its association with fish absence is unexpected, because gyrinid beetles are not thought to be susceptible to fish predation due to their predator defense mechanisms. We found four gyrinid species to be restricted to Maine's fishless lakes; however, we did not collect enough adults to determine if the association of *Dineutus* spp. with fish absence can be attributed to a single species most vulnerable to fish predation. Targeted collections of adult gyrinids might reveal whether one or more of the species restricted to our fishless study lakes would be useful as a more specific bioindicator.

The largest notonectid (*Notonecta insulata*) and corixid (*Hesperocorixa* spp.) taxa collected in our study were both identified as fishless bioindicators[1]. These taxa have been previously shown to be restricted to fishless habitats in eastern North America (Bendell & McNicol, 1987). *Notonecta insulata* is highly melanistic (Cook & Streams, 1984) and occupies sparsely vegetated open water habitats (Taylor, 1968; Streams & Newfield, 1972; Bennett & Streams, 1986), making it highly vulnerable to fish predation

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[1] Mean body size (mm) and standard deviation of commonly captured Hemiptera: Notonectidae - *Notonecta insulata* = 13.6 (1.1), *Notonecta undulata* = 12.0 (0.8); Corixidae - *Hesperocorixa* spp. = 10.5 (0.8), *Neocorixa* spp. = 8.3 (0.9), *Callicorixa* spp. = 8.0 (0.9), *Graptocorixa* spp. 7.8 (0.6), *Cenocorixa* spp. = 6.1 (0.9), *Palmacorixa* spp. = 5.5 (0.7), *Sigara* spp. = 5.7 (0.7)

(Cook & Streams, 1984). Fish predation on *Hesperocorixa* spp. is intense in open water habitats, but it can coexist with fish where thick *Sphagnum* provides refuge (Macan, 1976). In our study, *Hesperocorixa* spp. was found in a high proportion of fishless lakes (75%), especially fishless kettle lakes (100%), and a low proportion of fish-containing lakes (11%). Fish-containing lakes with *Hesperocorixa* spp. were rimmed with *Sphagnum* indicating that, while the distribution of this taxon is primarily related to fish absence, the availability of cover is also important. *Callicorixa* spp., another hemipteran identified as a fishless bioindicator, was found in 50% of fishless study lakes and was absent from fish-containing lakes. Although not exclusive to temporary waters (Savage, 1989), *Callicorixa* spp. are common in peatland pools (Morris, 1969) and temporary ponds (Williams, 1997), and species in this genus have previously been identified as indicators of temporary ponds in England (Collinson *et al.*, 1995). *Callicorixa* spp. prefers open water habitat and their predominance in temporary ponds likely is due to the absence of fish predation (Collinson *et al.*, 1995).

Chaoborus americanus has been shown to be intolerant of fish predation, and the inability of this species to coexist with fish has been widely documented (e.g. von Ende, 1979; Wissel *et al.*, 2003). Unlike other chaoborids that undergo diel vertical migration, *C. americanus* remains in the water column during the day and does not retreat to the sediments (von Ende, 1979). This species also is large and strongly pigmented, making it highly visible in the water column and vulnerable to fish predation (von Ende, 1979; Stenson, 1980). The presence of *C. americanus* mandibles in sediments has been used to infer long-term absence of fish (Lamontagne & Schindler, 1994; Uutala & Smol, 1996; Sweetman & Smol, 2006), including lakes in Maine (DeGoosh 2007; Schilling *et al.,*

2008*a*). Our macroinvertebrate surveys revealed that this species also is a good indicator of current fish absence.

3.5.4 Comparisons of submerged light trap and littoral sweep collections and their effectiveness for assessing fish absence

In the presence of fish, some taxa that otherwise would dwell in open water may restrict their habitat use to areas of cover (Macan, 1966; Macan, 1976; Luecke, 1986; Oscarson, 1987; Brett, 1989), reducing predation risk and enabling them to coexist with fish (Bennett & Streams, 1986). Comparisons of littoral sweeps and submerged light traps verified that differences in macroinvertebrate abundance and percent occurrence between fishless and fish-containing lakes were real and not due to differences in macroinvertebrate behavior in the two lake types. Although differences were not always significant in both sampling methods, patterns in taxonomic associations by lake type were consistent (Table A.1). We attribute the lack of significant differences in one sampling type vs. another to the effectiveness of each method for capturing the taxon in question. We found littoral sweeps more effective at capturing anisopterans, and possibly odonates in general (although we did not identify zygopterans collected in littoral sweeps). We found submerged light traps more effective at capturing Notonectidae and Coleoptera, indicating the effectiveness of this method for collecting free-swimming taxa. Highly mobile species may evade capture when approached with a net and are more effectively collected with passive capture modes (Hilsenhoff, 1987; Hilsenhoff, 1991; Streams, 1992; Hampton & Friedenberg, 2001). Studies that rely on sweep net collections to characterize macroinvertebrate assemblages indicate that such taxa are underrepresented (Fairchild *et al.*, 2000; Fairchild *et al.*, 2003).

Submerged light traps are an appropriate method to efficiently assess fish absence, because taxa selected as fishless bioindicators (Table 3.5) are active swimmers. The low-cost submerged light traps were more effective for collecting these taxa than littoral sweeps. Nocturnal deployment of submerged traps "baited" with a light source (i.e., glow sticks) likely enhanced the success of this collection technique for capturing fishless bioindicators, although the effect of light-baiting was not tested per se. Many taxa are more active at night, including several dytiscid genera that are thought to be primarily or totally nocturnal (Hilsenhoff, 1987), and many gyrinids that exhibit diurnal rafting behavior and forage singly at night (Heinrich & Vogt, 1980; Fitzgerald, 1987). Increased nocturnal activity generally is attributed to the avoidance of fish predation during light periods; however, macroinvertebrates in fishless lakes also exhibit diel changes in behavior and habitat use, with increased near-surface habitat use at night (Hampton & Friedenberg, 2001; Hampton & Duggan, 2003). The use of light in the traps is important, because light is an attractant to many aquatic insects, including corixids and notonectids (Hungerford *et al.*, 1955). A possible explanation for the success of our traps in capturing *Chaoborus* is that their zooplankton prey, such as *Daphnia*, are positively phototactic (Ringelberg, 1964) and may be concentrated in the traps. Hungerford *et al.* (1955) also collected *Chaoborus* in submerged traps illuminated with flashlights. Finally, submerged light traps are beneficial, because samples contain no debris or detritus and require minimal processing to extract specimens.

3.5.5 Implications for management

Schilling *et al.* (2008*a*) present a method to remotely predict the location of naturally fishless lakes with GIS and to assess the likelihood of historical fish absence

with paleolimnogical records in lake sediments. Here, we demonstrate a method to efficiently assess current fish absence with submerged light traps to catch fishless bioindicator taxa. Combined, these methods provide managers with tools to efficiently identify these habitats and to target their management and conservation activities.

Fishless and fish-containing lakes distributed across the state of Maine support vastly different macroinvertebrate communities. Maine's fishless lakes support greater macroinvertebrate abundance, species richness and several unique taxa. Fish introductions into these lakes likely would cause a significant loss of macroinvertebrate biodiversity locally at the lake scale, and potentially at larger scales in those landscapes where the abundance of naturally fishless lakes is low. Fish can lead to local extirpation of some taxa (Murdoch & Bence, 1987), a phenomenon that has been demonstrated by numerous small scale studies documenting the deleterious effects of fish introductions into previously fishless habitat on prey communities, including macroinvertebrates (Macan, 1976; Henrikson & Oscarson, 1978; Crowder & Cooper, 1982; Post & Cucin, 1984), other invertebrates (e.g. Brooks & Dodson, 1965; Taylor, 1968), as well as vertebrate prey (e.g. Werner & McPeek, 1994). Large scale studies have documented regional and range-wide declines of amphibians due to the loss of fishless lakes across the landscape (Bradford *et al.*, 1993; Knapp & Matthews, 2000; Pilliod & Peterson, 2001; Denoel *et al.*, 2005; Orizaola & Brana, 2006). The broad scale distribution of our study sites leads us to conclude that widespread fish introductions in Maine's naturally fishless lakes could lead to regional changes in native aquatic biodiversity and the decline of a unique aquatic natural ecosystem.

Conservation planning for naturally fishless lakes in the Northeast, however, lags behind the western USA, where restoration of stocked fishless lakes serves as a model for recovery of these unique habitats (Milliron, 1999; Knapp *et al.*, 2001; Hoffman *et al.*, 2004; Vredenburg, 2004; Knapp *et al.*, 2005; Yosemite National Park, 2006; Bunn *et al.*, 2007; Knapp *et al.,* 2007). Given the worldwide decline in fishless lakes due to both illegal fish stocking and that sanctioned by fisheries agencies (Donald, 1987; Bahls, 1992; Denoel *et al.*, 2005; Schilling *et al.*, 2008*a*), protection of fishless habitats that remain intact, and recovery of historically stocked lakes, should be conservation priorities for this resource.

3.6 Chapter acknowledgments

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4. EFFECTS OF INTRODUCED FISH ON NATIVE MACROINVERTEBRATES IN TWO TYPES OF HISTORICALLY FISHLESS LAKES

4.1 Abstract

Studies assessing effects of fish stocking on native biotic communities in historically fishless lakes have been limited to high-elevation headwater lakes stocked with non-native trout. Little is known about the effect of fish stocking in historically fishless lowland kettle lakes. We compared the effects of introduced fish on macroinvertebrate communities in kettle lakes stocked with centrarchids, salmonids, and cyprinids, and headwater lakes stocked with brook trout in Maine, USA. Fish had significant effects on macroinvertebrate community structure in both lake types, with reduced species richness and abundances of taxa characteristic of fishless lakes. The effects of fish were more pronounced in headwater lakes despite a less diverse introduced fish assemblage than in kettle lakes. We attribute this difference to abundant submerged vegetation and reduced stocking intensity in kettle lakes. We assessed effects of stocking duration on native macroinvertebrates in a subset of headwater lakes with known dates of trout introduction. Species richness and abundance of most taxa declined rapidly following trout introduction; however, richness and abundance were least in lakes with long stocking histories (≥ 40 years). Macroinvertebrates previously identified as fishless bioindicators were absent from all stocked lakes, indicating that trout rapidly eliminate these taxa. Conservation of this historically undervalued habitat requires protecting the remaining fishless lakes and recovering those that have been stocked.

4.2 Introduction

Naturally fishless lakes and their associated fauna represent a unique freshwater ecosystem type. Fishless lakes enhance regional species diversity by providing a unique freshwater habitat along a gradient of waterbody permanence and predator presence, ranging from temporary vernal pools lacking large dragonfly and fish predators to permanent lakes where fish are top predators (Wellborn *et al.*, 1996; Stoks & McPeek, 2003). Fishless lakes provide important prey items for migrating and breeding waterfowl (Bouffard & Hanson, 1997), passerines (P. Epanchin, personal communication) and reptiles (Matthews & Knapp, 2002). Historically, humans have undervalued the ecological importance of naturally fishless lakes, viewing them primarily as potential sport fish habitat or bait fish rearing sites. Widespread fish introductions have led to a worldwide decline in the number of fishless lakes and their associated communities (Donald, 1987; Bahls, 1992; Pister, 2001; Schilling *et al.*, 2008*a*). Rarefaction of this unique habitat type due to the introduction of predatory fish has been linked to landscape-scale losses of native prey species, including species of zooplankton (Stoddard, 1987; Bradford *et al.*, 1998; Knapp *et al.*, 2001), amphibians (Fisher & Shaffer, 1996; Bradford *et al.*, 1998; Knapp *et al.*, 2001; Pilliod & Peterson, 2001; Denoel *et al.*, 2005; Orizaola & Brana, 2006) and macroinvertebrates (Bradford *et al.*, 1998; Carlisle & Hawkins, 1998; Knapp *et al.*, 2001).

Studies documenting the detrimental effects of fish stocking in historically fishless lakes have focused on high-elevation headwater lakes stocked with non-native trout, primarily in western North America where fishless lakes historically were common (Donald, 1987; Bahls, 1992). This research is part of a larger body of work that questions

traditional fish management practices (Stanley, 1995; Rahel, 1997; Rahel, 2000). Recent research, also in western North America, has demonstrated the potential for native headwater lake fauna to recover following fish removal (Drake & Naiman, 2000; Donald *et al.*, 2001; Hoffman *et al.*, 2004; Knapp *et al.*, 2005; Knapp *et al.*, 2007). Recognizing the ecological value of fishless lakes and their potential for recovery has spurred state and federal agencies to take a more holistic management approach. Stocking high-elevation fishless lakes in western North America has been halted, and some lakes are being restored to their natural fishless condition (Milliron, 1999; Yosemite National Park, 2006; Bunn *et al.*, 2007).

There have been no similar attempts to evaluate or mitigate the effects of stocking historically fishless lakes in northeastern North America, a region where fish faunas are highly altered due to widespread introductions. While the predominant geographical trend of fish introductions in North America has been westward invasions of species native to the East, northeastern states contain some of the most altered fish faunas in the USA due to the low number of native species in this region considered desirable as game fish (Whittier & Kincaid, 1999; Rahel, 2000; Whittier, 2002). Fish have been moved liberally within their native ranges among eastern lakes, with many instances of translocations of "native" fish to waterbodies that have not previously contained these species (Whittier & Kincaid, 1999; Whittier, 2002). Many naturally fishless lakes in northeastern North America now contain fish (Schilling *et al.*, 2008*a*). These include headwater lakes stocked primarily with brook trout and kettle lakes stocked with a more diverse fish assemblage, including centrarchids, salmonids, and cyprinids (Schilling *et*

al., 2008*a*). The effects of fish stocking have never been studied in fishless kettle lakes, a physiographic lake type entirely different from headwater lakes.

The primary objective of this study was to compare effects of introduced fish on native macroinvertebrate communities in historically fishless headwater and kettle lakes in Maine, USA. Due to known effects of fish predation (Macan, 1965; Morin, 1984; Post & Cucin, 1984; Bendell & McNicol, 1987), we anticipated differences between macroinvertebrate communities of fishless and stocked lakes. We hypothesized that the effects of introduced fish on macroinvertebrate communities would differ between the two physiographic lake types, headwater and kettle, due to differences in lake habitat structure, stocking intensity, and fish species composition. Our second objective was to assess whether the effect of introduced fish on native macroinvertebrate communities in repetitively stocked lakes varies with the amount of time since the original fish introduction. We hypothesized that the effect of introduced fish on native macroinvertebrate communities would be more pronounced in lakes with long histories of repeated stocking than in recently stocked lakes.

4.3 Methods

4.3.1 Study design

We identified two physiographic types of naturally fishless lakes in two biophysical regions in Maine: headwater lakes in the central and western mountains and kettle lakes in the eastern lowlands and foothills (Schilling *et al.*, 2008*a*). Prior to being stocked, fish were naturally absent from these lakes since the last glaciation (10,000 years BP) created natural physical barriers to fish colonization (Schilling *et al.*, 2008*a*). Fishless lakes in western Maine are high-elevation headwater cirques isolated from fish

colonization by steep outlets impassable to fish. Fishless lakes in eastern Maine are kettle lakes formed in depressions left by glacial ice blocks. Many kettles have no surface water connections to other waterbodies and thus lack routes for fish movement. Additionally, many kettles are bog lakes with naturally low pH (Schilling *et al.,* 2008b), which limits fish species richness (Rahel, 1984).

We selected 16 currently fishless (eight headwater, eight kettle) and 14 historically fishless but now stocked (eight headwater, six kettle) lakes for study (Figure 4.1; Table A.4) by consulting fish survey records [Maine Department of Inland Fisheries and Wildlife (MDIFW), unpublished data] and a geographic information systems (GIS) analysis identifying lakes inaccessible to fish (Schilling *et al.*, 2008*a*). Historical fish survey records indicated that five of the stocked headwater lakes were fishless prior to state-authorized brook trout stocking (MDIFW, unpublished; Table A.4). The remaining three stocked headwater lakes and all six stocked kettle lakes were selected based on GIS analyses that demonstrated that physical characteristics of these lakes were similar to other known fishless lakes in the region (Schilling *et al.*, 2008*a*). Historical fishless status of these lakes was verified using paleolimnological analyses of *Chaoborus* remains in lake sediments (DeGoosh, 2007; Table A.4). The original date of fish introduction in these lakes is unknown, but sediment extractions indicate fish absence is estimated at 14-61 years before present (Davis *et al.*, 1994; DeGoosh, 2007).

Field surveys to describe fauna and habitat characteristics were conducted during single site visits made during summers 2002-2005. We qualitatively assessed habitat structure (visual assessment of the approximate amount of lake perimeter rimmed with littoral vegetation and distance it extended from shore), measured maximum lake depth

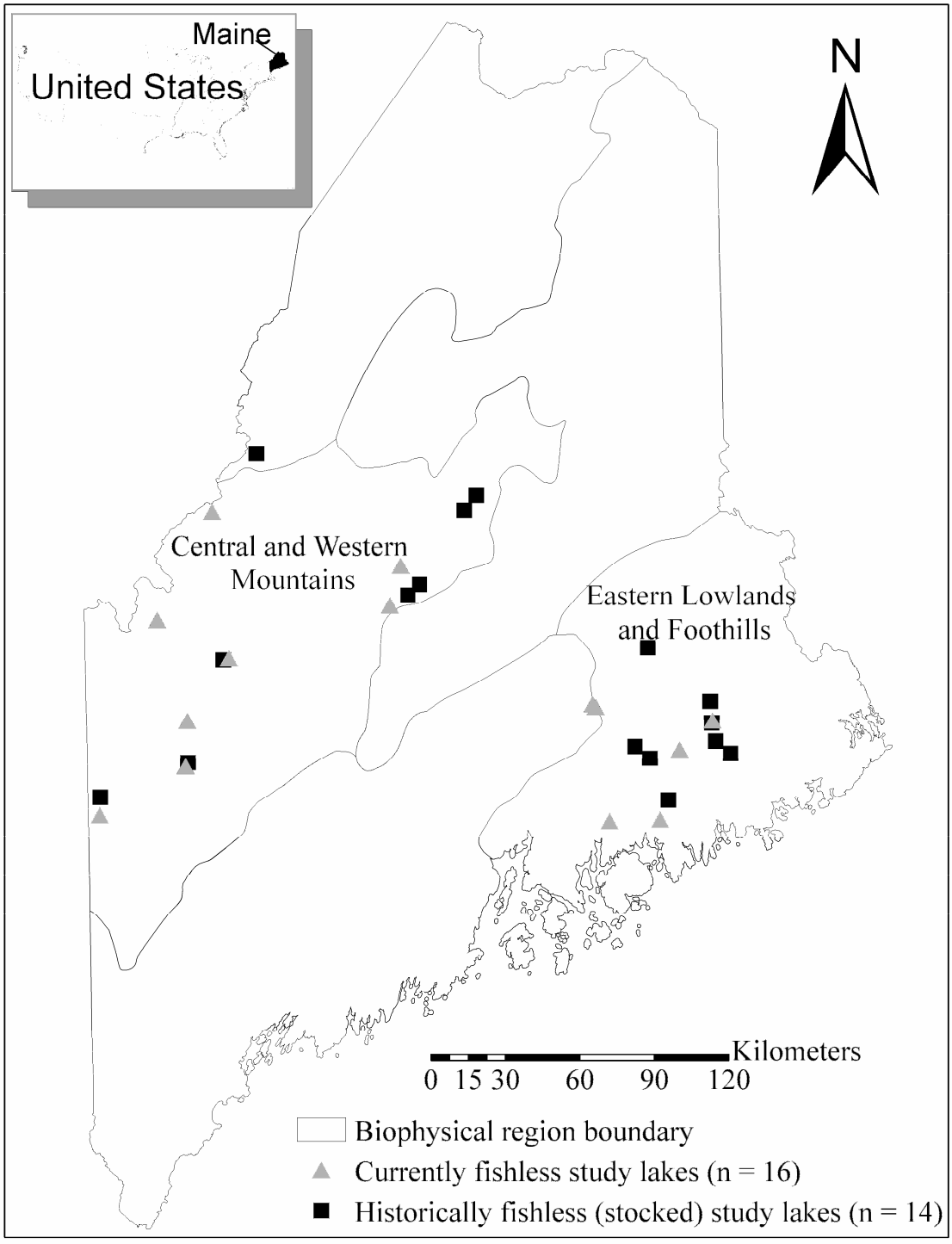

Figure 4.1. Distribution of fishless and stocked study lakes in Maine. Headwater lakes are located in the central and western mountains; kettle lakes are located in the eastern lowlands and foothills. Biophysical regions are from Krohn *et al.* (1999).

with a depth finder, and collected one water sample for closed cell pH analysis. Lake surface area and elevation were estimated with GIS. We assessed differences in measured physical variables between fishless and stocked lakes for each physiographic lake type, as well as between stocked headwater lakes and stocked kettle lakes, with Student's *t*-tests ($\alpha = 0.1$).

4.3.2 Fish and macroinvertebrate surveys

We verified fish absence (fishless lakes) and fish species composition (stocked lakes) with gillnets and minnowtraps (see Schilling *et al.*, 2008*b* for detailed methods). Macroinvertebrates were sampled overnight with submerged light traps (N = 10/lake) placed in the littoral zone and containing glow sticks to attract free swimming invertebrates (see Schilling *et al.*, 2008*b* for detailed methods). All samples were sieved (1mm mesh) and preserved (70% ethanol). Macroinvertebrates were counted and identified in the laboratory with a dissecting microscope. Depending on the life-stage and specimen condition, Hemiptera and Ephemeroptera were identified to genus, and Coleoptera, Odonata, Notonectidae, and *Chaoborus* spp. were identified to species based on primary literature (Table A.2).

4.3.3 Statistical analyses

We conducted separate statistical analyses for headwater and kettle lakes, testing for differences in macroinvertebrate communities between stocked lakes and fishless lakes, and then compared the results to assess whether the effect of introduced fish differed by physiographic lake type. We tested for differences in macroinvertebrate assemblage structure between fishless lakes and stocked lakes with multiresponse

permutation procedure (MRPP) and non-metric multidimensional scaling (NMS; PC-ORD version 5.0, MjM Software, Gleneden Beach, Oregon), performed on absolute abundances of common genera (occurring in > 10% of all study lakes). MRPP is a non-parametric method that tests for differences in species composition between two or more *a priori* defined groups (McCune & Grace, 2002). We report MRPP's chance-corrected within-group agreement value (*A*), which describes the effect size or degree of within-group homogeneity compared to the random expectation (i.e., *A* attains its maximum value of 1 when all items are identical within groups), and test statistic *T* (and its associated p-value), which describes separation between groups (i.e., the more negative the stronger the separation). We ran MRPP on a rank transformed distance matrix with the Sorensen distance measure and the n/sum(n) weighting factor (where n is the number of items in the group).

NMS is an ordination technique that uses rank order information in a dissimilarity matrix and is well-suited for community data, because it avoids the assumptions of normality and linearity (McCune & Grace, 2002). We ran NMS in autopilot mode ("slow and thorough" setting and Sorensen distance measure), finding the optimal dimensionality with 250 runs performed on real data followed by 250 runs with randomized data, with each run stepping down in dimensionality from six axes to one axis. The final ordination was obtained with the optimal dimensionality (n = 3 for both headwater and kettle lakes) and the best starting configuration as determined from previous runs. We performed five ordinations, evaluated each for consistency of interpretation, and chose the ordination with the lowest final stress value as the optimum solution. Differences in NMS scores between fishless lakes and stocked lakes were

analyzed with Student's *t*-tests ($\alpha = 0.1$). To simplify the graphical display, we plot the average position in ordination space of fishless and stocked lakes on the two axes that showed the strongest associations with fish presence.

We compared total and taxon-specific abundances (Student's *t*-tests; $\alpha = 0.1$) and the frequency of occurrence (Fisher's Exact Tests; $\alpha = 0.1$) of common macroinvertebrate taxa between fishless and stocked lakes. We also compared richness of families identified to species and genus richness for Corixidae (Students *t*-tests; $\alpha = 0.1$). We report results at the most aggregated taxonomic level with significant differences. Finer taxonomic levels are reported for taxa with genus or species associations with fishless or stocked lakes. We used SYSTAT to conduct these analyses (SYSTAT Software, version 11, Richmond, California). All abundance data were $\log_{10}(x + 1)$ transformed prior to analysis, and all data were summed across 10 light traps per lake.

To test whether the effect of introduced fish on native macroinvertebrate communities was more pronounced in lakes with long histories of repeated stocking than in recently stocked lakes, we analyzed data from a subset of headwater study lakes: three randomly selected fishless lakes and five stocked lakes with documented dates of original fish introduction [two stocked for three years prior (3yr duration) and three stocked for ~40 years (40yr duration) prior to our macroinvertebrate collections]. We compared macroinvertebrate abundance (total and taxon-specific) and total species richness in fishless, 3yr duration, and 40yr duration lakes with one-way ANOVA and Levene's test for equality of variances. Pairwise comparisons were made with Fisher's protected LSD when variances were equal and Games-Howell when variances were not equal ($\alpha = 0.1$). For 3yr duration and 40yr duration lakes we assessed presence/absence of six taxa

identified as bioindicators of fish absence in naturally fishless lakes (Schilling *et al.*, 2008*b*): *Graphoderus liberus, Dineutus* spp.*, Hesperocorixa* spp.*, Callicorixa* spp.*, Chaoborus americanus, Notonecta insulata*. These taxa demonstrate extremes of characteristics that increase invertebrate vulnerability to fish predation [e.g. large-bodied, active, conspicuous (Schilling *et al.*, 2008*b*)].

4.4 Results

4.4.1 Study lake characteristics

Headwater study lakes were small, high-elevation lakes (Table 4.1; Table A.4) with sparse aquatic vegetation or habitat structure in the littoral zone. Measured physical conditions (surface area, elevation, pH, maximum depth) did not differ between fishless and stocked headwater lakes. Kettle study lakes were small, low-elevation lakes (Table 4.1; Table A.4) with abundant *Sphagnum* mats along the shoreline and lake bottom. Fishless kettle lakes were more acidic than stocked kettle lakes ($t_{[12]} = -6.195$, $P < 0.001$). Stocked headwater and kettle lakes differed in elevation ($t_{[7]} = 7.688$, $P < 0.001$) and littoral vegetation structure. All stocked headwater lakes contained brook trout, which was the only fish species present in seven of these lakes (Table A.4). This species is stocked annually by airplane in six headwater lakes (MDIFW, unpublished). All stocked kettle lakes supported naturalized populations of one or more fish species, including salmonids, cyprinids, and centrarchids (Table A.4). Three kettle lakes are stocked regularly with brook trout (MDIFW, unpublished; Table A.4). The prevalence of non-game species in kettle lakes likely is due to the greater accessibility of these lakes to humans, resulting in bait fish introductions [as compared to headwater lakes, which are remote and primarily support state-managed game species (i.e., brook trout)].

Table 4.1. Physical characteristics [mean and standard error (SE)] of 30 historically fishless Maine lakes grouped by physiographic type and fish presence.

		Area (ha)	*Elevation (m)*	*pH*	*Max Depth (m)*
Headwater lakes	Fishless (n=8)	2.91 (0.65)	560.75 (75.38)	6.27 (0.26)	5.87 (1.45)
	Stocked (n=8)	3.31 (0.52)	744.38 (87.47)	6.11 (0.17)	7.09 (1.13)
Kettle lakes	Fishless n=8)	2.59 (0.79)	84.25 (9.15)	4.69 (0.14)	6.71 (1.81)
	Stocked (n=6)	3.21 (0.68)	71.33 (3.79)	6.13 (0.19)	4.57 (1.31)

4.4.2 Effects of stocked fish in headwater and kettle lakes

Macroinvertebrate community structure: Fishless and stocked lakes in both physiographic lake types supported distinct macroinvertebrate communities. MRPP indicated that homogeneity within fishless and stocked lakes was greater than compared to the random expectation ($A_{headwater} = 0.156$; $A_{kettle} = 0.255$) and that the difference between fishless and stocked lakes was significant for both headwater and kettle lakes ($T_{headwater} = -4.753$, $P < 0.001$; $T_{kettle} = -6.042$, $P < 0.001$). *A* was within the normal range (commonly <0.1 with values >0.3 considered fairly high) for ecological community data (McCune & Grace, 2002). Assemblages among fishless lakes were more similar than among stocked lakes, indicated by smaller average within group distances for fishless ($\mu_{headwater} = 0.234$; $\mu_{kettle} = 0.247$) than stocked lakes ($\mu_{headwater} = 0.610$; $\mu_{kettle} = 0.540$).

In both the headwater and kettle lake datasets, NMS identified three major gradients capturing most of the variability, with 87.2% ($r^2_{headwater}$ axis 1 = 0.476, axis 2 = 0.235, axis 3 = 0.162) and 93.1% (r^2_{kettle} axis 1 = 0.115, axis 2 = 0.152, axis 3 = 0.663) of the total variance explained. The final stress values ($stress_{headwater} = 8.560$; $stress_{kettle} = 6.386$) were small for ecological community analyses (typical range = 10 – 20); however, small stress values are expected, because stress is dependent on sample size, and we sampled a relatively small number of lakes (McCune & Grace, 2002). NMS plots of similarity in community composition showed strong clustering of lakes by fish presence

or absence (Figure 4.2A, B). NMS scores on axes 1 ($t_{[8]}$ = -3.296, P = 0.011) and 2 ($t_{[14]}$ = 2.621, P = 0.020) differed between fishless and stocked headwater lakes (Figure 4.2A). NMS scores on all three axes differed between fishless and stocked kettle lakes (axis 1: $t_{[12]}$ = 3.356, P = 0.006, axis 2: $t_{[12]}$ = 2.614, P = 0.023, axis 3: $t_{[12]}$ = -3.085, P = 0.009; Figure 4.2B). NMS scores were more variable among stocked lakes than fishless lakes, consistent with MRPP results, indicating greater variability in assemblage structure in stocked lakes than fishless lakes.

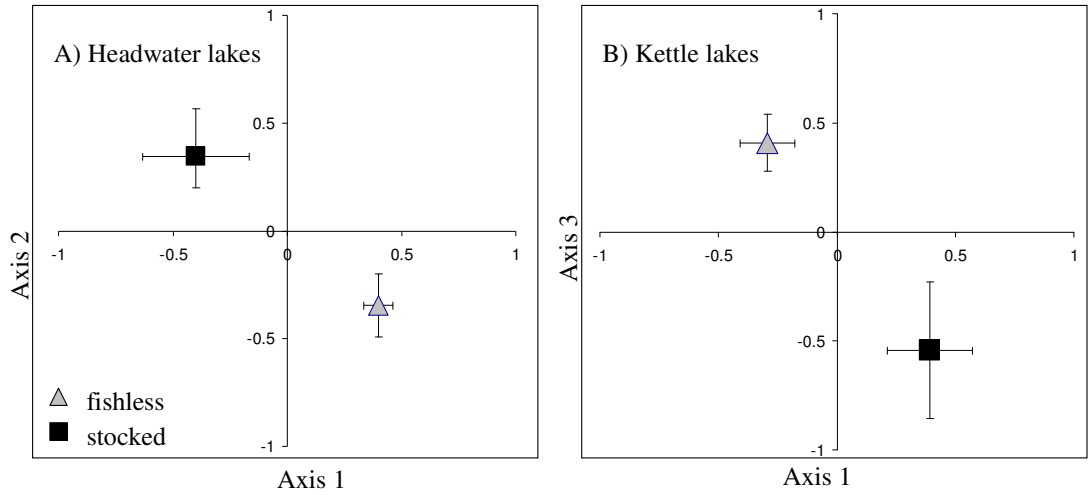

Figure 4.2. (A) For common macroinvertebrate taxa (present in >10% of lakes), mean NMS scores (± SE) for fishless and stocked headwater lakes on Axis 1 and Axis 2. Scores were derived from $\log_{10}(x+1)$ transformed abundances. (B) Same data as in (A) but for kettle lakes.

Taxon-specific analyses: Effects of introduced fish were more pronounced on macroinvertebrate communities in headwater lakes than in kettle lakes. The total number of macroinvertebrates captured was more than 1.5 times greater in fishless headwater lakes (μ = 2.61) than stocked headwater lakes (μ= 1.47; $t_{[14]}$ = -3.692, P = 0.002). Total abundance did not differ between fishless (μ = 2.586) and stocked kettle lakes (μ = 2.181, $t_{[12]}$ = -0.977, P = 0.348). More taxa differed in abundance or percent occurrence between fishless and stocked headwater lakes than fishless and stocked kettle lakes (Table 4.2).

Table 4.2. Mean abundance (standard error, SE) and percent occurrence of common taxa with differing distribution patterns between fishless (n = 8) and stocked (n = 8) headwater lakes and between fishless (n = 8) and stocked (n = 6) kettle lakes. Data are presented at the most aggregated taxonomic level with significant differences. Finer taxonomic levels are presented for taxa with genus or species associations with either lake type. Test statistics and p-values compare [$\log_{10}(x+1)$ transformed] abundance (Student's *t*-tests) and percent occurrence (Fisher's Exact Tests) between fishless and stocked lakes. Highlighted cells indicate significantly higher abundance and/or % occurrence in fishless (light grey) or stocked (dark grey) lakes. Slashed cells indicate no difference between fishless and stocked lakes.

	Headwater lakes								Kettle lakes							
	Percent occurrence			Abundance					Percent occurrence			Abundance				
Taxa	*Fishless*	*Stocked*	*p*	*Fishless mean (SE)*	*Stocked mean (SE)*	*t stat*	*df*	*p*	*Fishless*	*Stocked*	*p*	*Fishless mean (SE)*	*Stocked mean (SE)*	*t stat*	*df*	*p*
Hemiptera		ns		2.41 (0.25)	0.82 (0.31)	-3.990	14	0.001		ns			ns			
Notonectidae		ns		1.62 (0.40)	0.34 (0.21)	-2.870	14	0.012		ns		1.85 (0.33)	0.45 (0.17)	-3.437	12	0.005
Buenoa spp.		ns			ns				100.0	33.3	0.015	1.60 (0.38)	0.21 (0.16)	-3.39	9	0.008
Notonecta insulata	62.5	0.0	0.026	0.64 (0.24)	0.00 (0.00)	-2.713	7	0.030	50.0	0.0	0.085	0.40 (0.20)	0.00 (0.00)	-2.27	7	0.057
Corixidae		ns		2.13 (0.23)	0.78 (0.29)	-3.680	14	0.003		ns			ns			
Callicorixa spp.	62.5	0.0	0.026	0.41 (0.14)	0.00 (0.00)	-2.890	7	0.023		ns			ns			
Cenocorixa spp.		ns			ns				25.0	100.0	0.010	0.16 (0.12)	1.42(0.36)	3.32	6	0.016
Hesperocorixa spp.	50.0	0.0	0.077	0.54 (0.23)	0.00 (0.00)	-2.346	7	0.051	100.0	33.3	0.015	0.81 (0.15)	0.16 (0.10)	-3.35	12	0.006
Neocorixa spp.	50.0	0.0	0.077	0.58 (0.27)	0.00 (0.00)	-2.166	7	0.067		ns			ns			
Sigara spp.	87.5	12.5	0.010	0.91 (0.19)	0.04 (0.04)	-4.459	8	0.002		ns			ns			
Coleoptera	100.0	50.0	0.077	1.67 (0.21)	0.33 (0.15)	-5.160	14	0.000		ns		1.89 (0.53)	0.73 (0.42)	-3.38	12	0.006
Gyrinidae		ns		0.98 (0.20)	0.24 (0.11)	-3.236	14	0.006		ns			ns			
Dineutus spp.	62.5	0.0	0.026	0.50 (0.15)	0.00 (0.00)	-3.307	7	0.013		ns			ns			
Gyrinus spp.		ns		0.78 (0.22)	0.22 (0.10)	-2.293	10	0.045		ns			ns			
Dytiscidae	87.5	25.0	0.041	1.44 (0.27)	0.13 (0.09)	-4.485	8	0.002	100.0	50.0	0.055	1.76 (0.26)	0.24 (0.14)	-4.69	12	0.001
Dytiscus spp.		ns		0.43 (0.22)	0.00 (0.00)	-1.990	7	0.087		ns			ns			
Graphoderus liberus	87.5	0.0	0.001	0.96 (0.18)	0.00 (0.00)	-5.512	7	0.001	100.0	0.0	0.000	1.21 (0.30)	0.00 (0.00)	-4.70	7	0.002
Ilybius spp.		ns			ns					ns		0.11 (0.06)	0.00 (0.00)	-2.05	7	0.080
Haliplidae	75.0	12.5	0.041	0.36 (0.10)	0.09 (0.09)	-2.077	14	0.057		ns			ns			
Odonata		ns			ns					ns			ns			
Aeshnidae		ns		1.44 (0.12)	0.06 (0.06)	-4.838	14	0.000		ns			ns			
Aeshna spp.		ns		0.30 (0.11)	0.06 (0.06)	-1.879	14	0.081		ns		0.55 (0.21)	0.13 (0.09)	-1.88	9	0.092
Aeshna eremita		ns		0.21 (0.09)	0.04 (0.04)	-1.831	10	0.097		ns			ns			
Libellulidae	50.0	0.0	0.077	0.63 (0.26)	0.00 (0.00)	-2.413	7	0.047		ns			ns			
Leucorrhinia spp.	50.0	0.0	0.077	0.62 (0.26)	0.00 (0.00)	-2.391	7	0.048		ns			ns			
Chaoboridae		ns		0.94 (0.16)	0.44 (0.20)	-1.945	14	0.072		ns			ns			
Chaoborus americanus	62.5	0.0	0.026	0.48 (0.16)	0.00 (0.00)	-2.986	7	0.020	62.5	0.0	0.031	0.60 (0.21)	0.00 (0.00)	-2.887	7	0.023
Chaoborus punctipennis		ns			ns					ns		0.15 (0.13)	1.38 (0.48)	2.522	6	0.045

Eight families (Figure 4.3) and 15 genera and species (Table 4.2) were more abundant in fishless headwater lakes than stocked headwater lakes. Two families (Figure 4.3) and 10 genera and species (Table 4.2) were more abundant in fishless kettle lakes than stocked kettle lakes. No taxa were more abundant in stocked than fishless headwater lakes. *Cenocorixa* spp. and *C. punctipennis* were more abundant in stocked than fishless kettle lakes. Dytiscid beetles were strongly associated with fish absence in both headwater and kettle lakes, with *Graphoderus liberus* abundant in the majority of fishless lakes but absent in stocked lakes. *Dytiscus* spp. were more abundant in fishless than stocked headwater lakes, and *Ilybius* spp. were more abundant in fishless than stocked kettle lakes Gyrinid and haliplid beetles were strongly associated with fishless headwater lakes, with *Dineutus* spp., and *Gyrinus* spp. more abundant in fishless lakes. Notonectids were strongly associated with fish absence in both headwater and kettle lakes, with *Notonecta insulata* abundant in the majority of fishless lakes but absent in stocked lakes. *Buenoa* spp. was more abundant and occurred more frequently in fishless than stocked kettle lakes. Corixids were strongly associated with fish absence in headwater lakes, with *Callicorixa spp, Hesperocorixa* spp., *Neocorixa* spp., and *Sigara* spp. more abundant and occurring more frequently in fishless lakes. *Hesperocorixa* spp. was the only corixid more abundant in fishless than stocked kettle lakes. The chaoborid *Chaoborus americanus* was strongly associated with fish absence in both headwater and kettle lakes and was abundant in the majority of fishless lakes but absent in stocked lakes. The odonates *Aeshna eremita* and *Leucorrhinia* spp. were more abundant in fishless than stocked headwater lakes. No odonates differed in abundance or percent occurrence in kettle lakes.

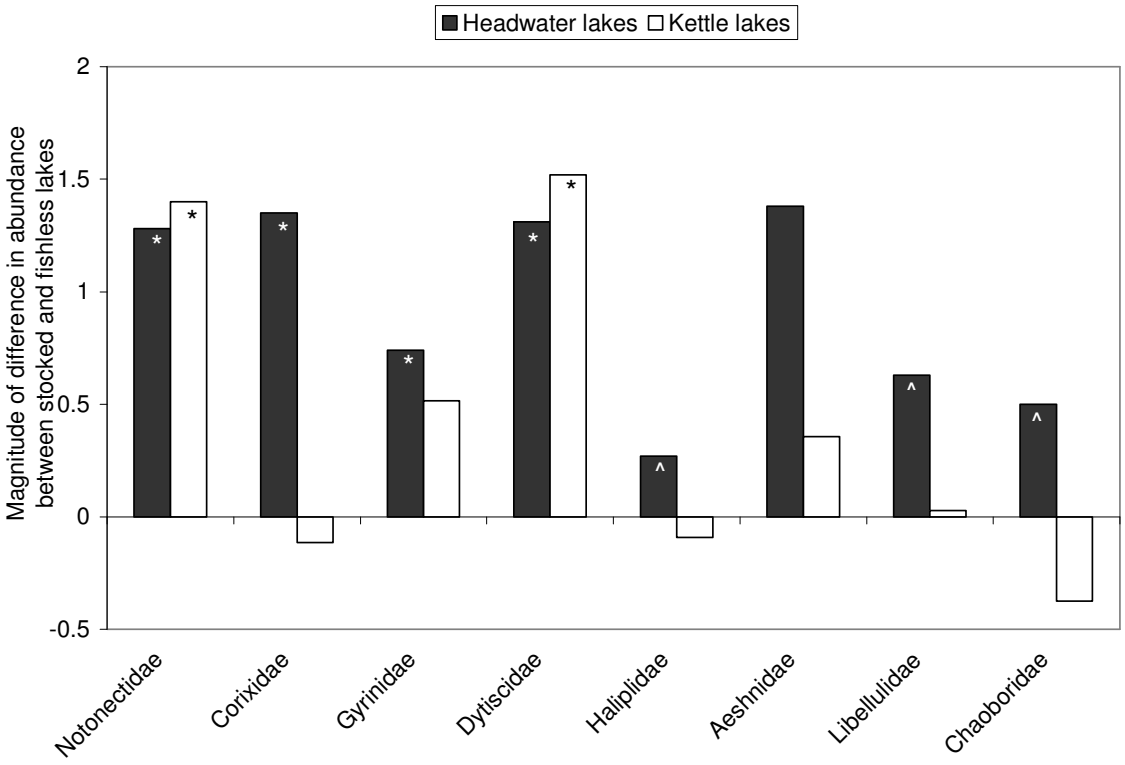

Figure 4.3. Magnitude of difference in abundance [$\log_{10}(x + 1)$ transformed] of macroinvertebrate families collected in fishless and stocked headwater and kettle lakes. Symbols inside bars indicate significant differences within each physiographic lake type between fishless and stocked lakes; significant differences indicated by ^ (P<0.1), * (P<0.05), ** (P<0.01), and *** (P<0.001).

Average species richness of all identified species was more than three times greater in fishless headwater lakes than stocked headwater lakes ($\mu_{fishless} = 8.4$, $\mu_{stocked} = 2.6$; $t_{[14]} = -3.157$, P = 0.007). Average species richness was greater in fishless kettle lakes than stocked kettle lakes ($\mu_{fishless} = 9.4$, $\mu_{stocked} = 5.2$; $t_{[12]} = -2.520$, P = 0.026), although the magnitude of difference was less in kettle lakes than in headwater lakes. In particular, Dytiscidae richness was markedly greater in both fishless headwater ($\mu_{fishless} = 2.4$, $\mu_{stocked} = 0.0$; $t_{[7]} = -3.800$, P = 0.007) and fishless kettle ($\mu = 2.5_{fishless}$, $\mu_{stocked} = 0.3$; $t_{[9]} = -3.800$, P = 0.004) lakes. Corixidae demonstrated the highest richness (8) at the genus level in our collections (*Arctocorixa, Callicorixa, Cenocorixa, Graptocorixa, Hesperocorixa,*

Neocorixa, Palmacorixa, Sigara), and fishless headwater lakes contained >4 times more genera of Corixidae than stocked headwater lakes ($\mu_{fishless} = 3.3$, $\mu_{stocked} = 0.5$; $t_{[14]} = -4.075$, $P = 0.001$). Corixidae genus richness did not differ in kettle lakes ($\mu_{fishless} = 2.6$, $\mu_{stocked} = 2.5$; $t_{[12]} = -0.139$, $P = 0.892$).

4.4.3 Effects of stocking duration on macroinvertebrate community structure

Stocking duration had a significant effect on total macroinvertebrate abundance: all pairwise comparisons of total macroinvertebrate abundance were significantly different between fishless, 3yr duration, and 40yr duration lakes (Figure 4.4). Macroinvertebrate faunas were more depauperate in 40yr duration than 3yr duration lakes, with nine of 15 families present in the fishless lakes subset absent from 40yr duration lakes, compared to three families absent from 3yr duration lakes. Dytiscidae, Notonectidae, and Haliplidae abundances were lower in 3yr duration than fishless lakes. Dytiscidae and Haliplidae abundances were similar in 3yr and 40yr duration lakes; whereas, Notonectidae abundance was lower in 40yr duration than 3yr duration lakes. Corixidae abundance was similar in fishless and 3yr duration lakes but was lower in 40yr duration lakes than both fishless and 3yr duration lakes. Average species richness was less three years after fish introduction (i.e., significant difference between fishless and 3yr duration lakes) and remained low in 40yr duration lakes (Table 4.3). Five of six fishless bioindicator taxa (*Graphoderus liberus, Dineutus* spp.*, Callicorixa* spp.*, Chaoborus americanus, Notonecta insulata*) were present in at least two fishless lakes and were absent from 3yr duration and 40yr duration lakes. One bioindicator taxon, *Hesperocorixa* spp.*,* was absent from the fishless lakes subset, as well as 3yr duration and 40yr duration lakes.

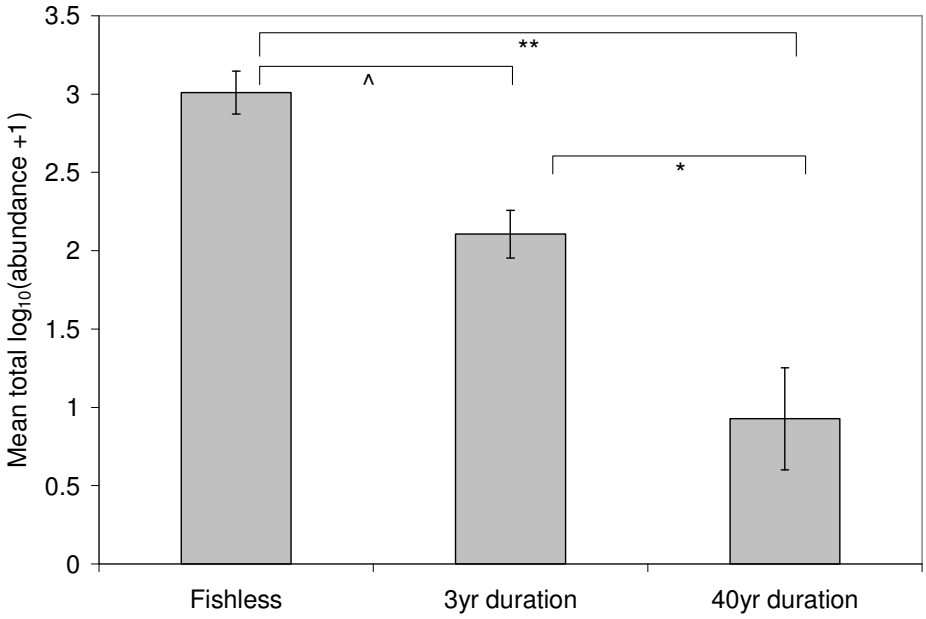

Figure 4.4. Mean (±SE) total abundance of macroinvertebrates in fishless headwater lakes (n = 3) and headwater lakes stocked for 3 years (3yr duration; n = 2) and 40 years (40yr duration; n = 3) prior to our study; significant differences indicated by ^ (P = 0.055), * (P = 0.023), and ** (P = 0.001).

Table 4.3. Taxa with significant differences in abundance and species richness in fishless lakes (n = 3), lakes stocked three years prior (3yr duration; n = 2) and lakes stocked ~40 years prior (40yr duration; n = 3) to our macroinvertebrate collections. Pairwise comparisons were made with ANOVA with Fisher's protected LSD or Games-Howell.

	Fishless mean (SE)	*3yr duration mean (SE)*	*40yr duration mean (SE)*	*F ratio*	*p*	*Fishless v 3yr duration*	*Fishless v 40yr duration*	*3yr duration v 40yr duration*
Total abundance	3.01 (0.14)	2.11 (0.15)	0.93 (0.33)	20.537	0.004	0.055	0.001	0.023
Hemiptera	2.84 (0.19)	1.86 (0.18)	0.10 (0.10)	85.205	0.000	0.009	0.000	0.001
Corixidae	2.37 (0.35)	1.70 (0.11)	0.10 (0.10)	24.689	0.003	0.240	0.000	0.000
Notonectidae	2.47 (0.28)	1.20 (0.42)	0.00 (0.00)	27.533	0.002	0.042	0.002	0.052
Coleoptera	1.89 (0.26)	0.66 (0.18)	0.10 (0.10)	22.909	0.003	0.009	0.001	0.122
Dytiscidae	1.73 (0.26)	0.15 (0.15)	0.00 (0.00)	28.349	0.002	0.012	0.041	0.690
Haliplidae	0.67 (0.03)	0.00 (0.00)	0.00 (0.00)	332.746	0.000	0.004	0.004	na
Species richness	12.33 (0.33)	3.50 (1.50)	0.67 (0.33)	93.286	0.000	0.000	0.000	0.203

4.5 Discussion

Introducing fish into fishless headwater and kettle lakes precipitates dramatic changes in macroinvertebrate community structure, with rarefaction and elimination of several taxa. Our results agree with previous studies on the effects of introduced fish on macroinvertebrates in historically fishless lakes (Luecke, 1990; Liss *et al.*, 1995; Bradford *et al.*, 1998; Carlisle & Hawkins, 1998; Knapp *et al.*, 2001; Knapp *et al.*, 2005), which has been limited to headwater lakes stocked with trout. Our results extend beyond previous findings to show that negative effects of introduced fish occur in kettle lakes stocked with a wider diversity of fish species, but the effects of introduced fish are more pronounced in headwater lakes stocked only with trout, with long term repetitive stocking exacerbating predatory effects.

Fishless kettle and headwater lakes support similar macroinvertebrate communities, despite differences in lake physical characteristics (i.e., elevation, pH, habitat structure; (Schilling *et al.*, 2008*b*). Both lake types support taxonomically-rich macroinvertebrate communities, with abundant populations of large-bodied, active, conspicuous invertebrates (Schilling *et al.*, 2008*b*). Although the pre-stocking faunal composition of kettle and headwater lakes may be similar, macroinvertebrate communities in stocked headwater lakes deviate more from the original fishless condition than in stocked kettle lakes. Stocked headwater lakes have greatly reduced abundances of taxa that characterize fishless lake macroinvertebrate communities (e.g. Notonectidae, Corixidae, Dytiscidae, Aeshnidae, Libellulidae, Chaoboridae), with some taxa eliminated completely (e.g. fishless bioindicator taxa). Fewer of these taxa were affected by introduced fish in kettle lakes (Table 4.2).

Three non-mutually exclusive hypotheses may explain the strong effect of introduced trout in our headwater study lakes. First, the insularity of headwater lakes may limit macroinvertebrate dispersal, resulting in local adaptation and inability to respond when fish are introduced. Second, headwater fishless lakes are structurally simple systems compared with kettle lakes, which may make their native fauna particularly vulnerable to fish predation. Finally, repeated stocking of trout in headwater lakes may exacerbate their effects on native fauna.

4.5.1 Non-native fish in isolated lakes

Strong effects of trout in headwater lakes may be due to the inability of macroinvertebrate prey to respond appropriately to predators with which they do not coexist (McPeek, 1990*b*; McIntosh & Townsend, 1994; Caudill & Peckarsky, 2003; Stoks *et al.*, 2003). While studies have found avoidance responses to fish by invertebrate prey in fishless streams (Tikkanen *et al.*, 1996; McIntosh & Peckarsky, 1999), headwater lake invertebrates may be less responsive to changes in predator regimes. Aerial dispersal of adults between fishless and fish-containing streams may be key to maintaining flexible predator avoidance behavior (Tikkanen *et al.*, 1996). When invertebrate dispersal is limited, however, flexible avoidance behaviors may be lost as consecutive generations of potential prey experience the same predation regime (Abjornsson *et al.,* 2004). Although many of the taxa affected by fish introductions in headwater lakes have winged adult stages, headwater lakes are cirques in constrained basins where topographic barriers and disjunction from wetlands may impede dispersal to habitats containing fish (Schilling *et al.*, 2008*a*). Limited dispersal would result in local adaptation and the inability to respond when fish are introduced.

4.5.2 Structural complexity

Structural complexity created by submergent vegetation can weaken the top-down effects of fish on macroinvertebrates (Crowder & Cooper, 1982; Gilinsky, 1984; Diehl, 1992; Carlisle & Hawkins, 1998). Therefore, stronger effects of fish on prey communities in headwater lakes with simple habitat structure, and weaker effects in kettle lakes with complex habitat structure should be anticipated. Extensive littoral and benthic *Sphagnum* mats typical of kettle lakes likely provide macroinvertebrates refuge from fish predation (Henrikson, 1993). Our results are consistent with studies in western North America that document the effectiveness of introduced trout as littoral predators in historically fishless high-elevation lakes with simple habitat structure compared to lower elevation sites with more complex habitat structure (Knapp *et al.* 2005).

4.5.3 Stocking intensity

Stocking intensity is greater in headwater than kettle lakes. Most fish populations in kettle lakes are naturalized and not supplemented by stocking. The majority of headwater lakes are stocked annually with brook trout, based on the assumption that winterkills and lack of suitable spawning habitat prevent fish from persisting in these lakes. Similar assumptions commonly are made by fisheries managers throughout the western USA and seem to result in frequent stocking of self-sustaining trout populations (Bahls, 1992). Because predation pressure increases with fish density (Pierce & Hinrichs, 1997), regularly stocking lakes that also may have reproducing trout populations likely intensifies the effects on native fauna. Continual stocking of trout in high-elevation lakes exacerbates their effect on ecosystem processes (Schindler *et al.*, 2001), and historically fishless lakes with high densities of introduced trout exhibit stronger negative effects of

fish predation on native macroinvertebrates than lakes with lower fish densities (Knapp *et al.*, 2005). Thus, high fish densities in regularly stocked lakes may explain the stronger effect of introduced fish in headwater lakes than kettle lakes, despite higher fish species richness in kettle lakes.

The hypothesis that stocking intensity may explain, in part, the stronger effect of introduced fish in headwater lakes is supported by our result that the longer fish are repeatedly stocked in headwater lakes, the more dramatic the effects on native fauna. Fishless bioindicators were not present in our headwater lakes after only three years of stocking. Yet, the most pronounced effects of fish on native macroinvertebrates, in terms of reductions in abundance and elimination of taxa, were observed in lakes with long stocking histories. This suggest that while the largest, most conspicuous macroinvertebrates are affected quickly by fish, others are affected as fish become more food-limited. Thus, lake communities are perturbed soon after fishless lakes are stocked for the first time, yet continued stocking exacerbates the original effects (see also Schindler *et al.* 2001).

4.5.4 Management implications

Fishless lakes support unique macroinvertebrate communities and play a critical role in maintaining aquatic biodiversity across the landscape (Knapp *et al.*, 2001; Stoks & McPeek, 2003; Schilling *et al.*, 2008*b*). Our study demonstrates the deleterious effects of introduced fish on these increasingly rare freshwater ecosystems and shows that communities in headwater lakes with long stocking histories are particularly vulnerable. The potential for recovery of native faunal assemblages declines the longer fish are present and the more widespread the spatial extent of stocking due to loss of

recolonization (Bradford *et al.*, 1993). Given the declining number of these habitats throughout northeastern North America (Schilling *et al.*, 2008*a*) and elsewhere (Donald, 1987; Bahls, 1992; Pister, 2001; Denoel *et al.*, 2005) and their role in maintaining biodiversity, historically fishless lakes should be prioritized for conservation, with particular concern for headwater lakes with long stocking histories. Conservation planning for naturally fishless lakes in northeastern North America lags behind that in western North America, where restoring stocked fishless lakes serves as a model for recovery of these systems (Milliron, 1999; Hoffman *et al.*, 2004; Yosemite National Park, 2006; Bunn *et al.*, 2007; Knapp *et al.*, 2007). A key obstacle in protecting these habitats is that agencies responsible for their conservation often also are charged with increasing opportunities for resource use by maintaining and expanding fish stocking. Agencies need to resolve these conflicting management goals and to adopt strategies that protect these unique and increasingly rare habitats.

4.6 Chapter acknowledgements

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5. NOVEL CONTRIBUTIONS AND MANAGEMENT RECOMMENDATIONS

5.1 Introduction

This study was developed to gain a better understanding of the landscape (i.e., geographic distribution) and local (i.e., native communities) characteristics of currently and historically fishless lakes in Maine, with the goal of providing the Maine Department of Inland Fisheries and Wildlife (MDIFW) information and tools to plan conservation of this resource. I identified two physiographic fishless lake types located primarily in two biophysical regions (Figure 2.1; Krohn *et al.*, 1999): kettle lakes in the eastern lowlands and foothills and headwater lakes in the central and western mountains. These lakes have been fishless since natural physical barriers to fish colonization were created by the last glaciation (10,000 years BP). Kettle lakes formed in depressions left by glacial ice blocks. Many kettles have no surface water connections to other waterbodies and thus lack routes for fish movement. Additionally, many kettles are bog lakes with naturally low pH, which limits fish species richness (Rahel, 1984). In contrast, fishless lakes in Maine's central and western mountains are high elevation headwater cirques isolated from fish colonization by steep outlets impassable to fish.

The objectives of this research were: 1) develop a quantitative method to remotely detect naturally fishless headwater and kettle lakes in Maine, 2) conduct a landscape-scale assessment of unique attributes of fishless headwater and kettle lake macroinvertebrate communities, 3) identify macroinvertebrate bioindicators to efficiently assess fish absence, and 4) assess the effects of fish introductions by studying invertebrate communities in historically fishless headwater and kettle lakes.

5.2 Key Findings

5.2.1 Predicting the locations of Maine's fishless lakes

I identified geomorphic and geographic factors (e.g. connectivity, surrounding slope) correlated with fish absence in 30 known fishless lakes in Maine (24 western headwater lakes and 6 eastern kettle lakes) with GIS. Fish absence from western lakes was related to elevation (+), minimum percent slope in the 500 m buffer (+), maximum percent slope in the 500 m buffer (+) and percent cover of herbaceous-emergent wetland in 1000 m buffer (-). Fish absence from eastern lakes was related to the lack of a stream within 50 m of the lake. Based on these factors, I developed a logistic regression model for each region estimating the likelihood of fish absence in potentially fishless lakes [i.e., waterbodies with 0.6–10.1 ha surface area (the size of Maine's documented fishless lakes and minimum size detectible on Maine's lake polygon coverage) located in the catchments where fishless lakes have been documented; Figure 2.4].

The models predicted that 101 study lakes were historically fishless in addition to the 30 known fishless lakes used for model building, with the eastern region hosting a greater proportion than the western region (Table 2.2). Therefore, I predicted that of the 3,281 eastern and western lakes I identified in the 0.6–10.1 ha size range, 4.0% are naturally fishless. My estimates are likely conservative, however, because within-lake conditions that might further limit fish presence (e.g., low pH, low water depth) are not included in the models. I verified model predictions by assessing current fish absence (with fish surveys) or historical fish absence (with paleolimnological analysis of lake sediments) in 21 (9 headwater, 12 kettle) lakes predicted to be fishless. Models correctly predicted 76% (7 headwater, 9 kettle) of lakes to be historically fishless (Table 2.4). Of

these, 66% (4 headwater, 6 kettle) currently contain fish and thus have been subject to undocumented fish introductions. Additionally, 7 of 30 historically fishless lakes used for model building have been stocked within the past 50 years. Extrapolating these results, ~107 (30 model building lakes + 76% of 101 predicted lakes) of Maine's 3,281 central and western mountains and eastern lowlands and foothills lakes (0.6 -10.1ha) were historically fishless; ~58 (7 model building lakes + 66% of 77 predicted lakes) of these now contain fish and 57 (23 model building lakes + 44% of 77 predicted lakes) remain in their natural fishless state.

5.2.2 Maine's fishless lake macroinvertebrate communities

Comparisons of macroinvertebrate communities in fishless kettle and headwater lakes revealed few differences between the two physiographic fishless lake types (Table 5.1); in contrast, comparisons of fishless and fish-containing lake communities revealed numerous differences in macroinvertebrate community structure, abundance, taxonomic composition and species richness (Table 5.1; Schilling *et al.*, 2008*b*). Thus, Maine's naturally fishless lakes support unique macroinvertebrate communities compared to similar lakes containing fish, and fish presence or absence in lakes is a stronger determinant of community structure than lake origin and physiography. Comparisons of macroinvertebrate communities in currently fishless and historically fishless lakes show that introducing fish into naturally fishless lakes results in dramatic changes in macroinvertebrate community structure, with rarefaction and elimination of several taxa (Schilling *et al.*, in prep).

Table 5.1 Outline of key findings from comparisons of macroinvertebrate communities (sampled with littoral sweep and submerged aquatic light traps) between: 1) fishless headwater (n = 8) and fishless kettle (n = 8) lakes; 2) fishless (n = 16) and fish-containing (n = 18) lakes; 3) fishless (n = 16) and historically (now stocked) fishless (n = 14) lakes.

1)	**Communities in fishless kettle and headwater lakes *were not* distinct.**
a)	The two lake types were similar in total macroinvertebrate abundance, total species richness, and richness at the family level.
b)	Of 46 collected taxa (Table 3.2), 5 differed between the two lake types (Figure 3.3)
2)	**Communities in fishless lakes and fish-containing lakes *were* distinct.**
a)	Average species richness was > 2x greater in fishless than fish-containing lakes, with 6/10 families more speciose in fishless lakes. Corixidae richness at the genus level was 1.5x greater in fishless than fish-containing lakes.
b)	Total macroinvertebrate abundance was 1.5x greater in fishless than fish-containing lakes, with greater abundances of Hemiptera (True Bugs), Coleoptera (Beetles) and Odonata (Damselflies and Dragonflies) in fishless lakes.
c)	Many taxa were significantly associated with fishless lakes (Figure 3.7).
i)	Taxa more abundant and more frequently captured in fishless lakes: Notonectidae (Backswimmers) and Corixidae (Waterboatmen; *Buenoa* spp., *Notonecta insulata*, *Callicorixa* spp., *Hesperocorixa* spp., and *Sigara* spp.), Gyrinidae (Whirligig Beetles) and Dytiscidae (Predaceous Water Beetles; *Dineutus* spp., *Gyrinus* spp., *Graphoderus liberus*, *Ilybius* spp., and *Thermonectes* spp.), Aeshnidae (Darners; *Aeshna eremita*), Libellulidae (Skimmers; *Leucorrhinia glacialis*), and Coenagrionidae (Pond Damselflies)
ii)	Fishless bioindicators: Six taxa (*Chaoborus americanus, Notonecta insulata, Graphoderus liberus, Callicorixa* spp., *Hesperocorixa* spp. and *Dineutus* spp.) were absent or occurred rarely in fish-containing lakes but were widespread among fishless lakes
d)	Few taxa were associated with fish-containing lakes (Figure 3.7).
i)	No families were significantly more speciose in fish-containing lakes than in fishless lakes.
ii)	Gerridae (Water Striders) was the only common taxon restricted to fish-containing lakes, and *Cenocorixa* spp. and *Chaoborus punctipennis* were more abundant and occurred more frequently in fish-containing lakes.
3)	**Introduced fish affected macroinvertebrate community structure in both fishless lake types, with effects more pronounced in headwater lakes.**
a)	Total macroinvertebrate abundance was > 1.5x greater in fishless headwater lakes than stocked headwater lakes, but did not differ between fishless and stocked kettle lakes.
b)	Eight families and 15 genera/species were more abundant in fishless than stocked headwater lakes. Two families and 10 genera/species were more abundant in fishless than stocked kettle lakes (Table 4.2).
c)	No taxa were more abundant in stocked than fishless headwater lakes. Two taxa, *Cenocorixa* spp. and *C. punctipennis,* were more abundant in stocked than fishless kettle lakes.
d)	Average species richness was > 3x greater in fishless than stocked headwater lakes and was 1.8x greater in fishless than stocked kettle lakes.
i)	Dytiscidae richness was ~8x greater in fishless than stocked lakes in both lake types
ii)	Corixidae richness at the genus level was > 4x greater in fishless than stocked headwater lakes, but did not differ in kettle lakes.
e)	Headwater lakes with long stocking histories (40yr) showed more dramatically altered communities than recently stocked (3yr) lakes (Figure 4.4).

5.2.3 Ecological effects of stocking Maine's fishless lakes

The landscape and local effects of stocking naturally fishless lakes in eastern North America were unknown prior to this study. Loss of fishless lakes due to fish introductions into lakes across the landscape and detrimental effects of introduced fish on native macroinvertebrate communities in Maine are consistent with studies in other regions [e.g. western North America (Donald, 1987; Bahls, 1992; Pister, 2001)]. Effects of stocking have not previously been reported in fishless kettle lakes, a physiographic lake type that occurs not only in New England but also throughout formerly glaciated regions of North America. Negative effects of introduced fish occur in kettle lakes stocked with a diverse fish assemblage, however, the effects of introduced fish are more pronounced in headwater lakes stocked only with trout. Long term repetitive stocking exacerbates predatory effects (Schilling *et al.*, in prep).

Although studies comparing macroinvertebrate communities in fishless and fish-containing lakes in eastern North America are relatively numerous, few have been conducted at the landscape scale, and most have focused on lakes that lost their fish populations due to acidification (Bendell, 1986; Bendell & McNicol, 1987; Bendell & McNicol, 1995; Strong & Robinson, 2004; Arnott & Jackson, 2006). Results from these and other studies consistently show that the distribution and abundance of many lake-dwelling aquatic insects are driven primarily by the occurrence of fish predators rather than differences in lake environmental variables, such as pH (Eriksson *et al.*, 1980; Bendell, 1986; Bendell & McNicol, 1987; McNicol *et al.*, 1987; Brett, 1989; McPeek, 1990*a*; Arnott & Jackson, 2006) and habitat structure (Bennett & Streams, 1986; Binckley & Resetartiz, 2005). This strong fish effect is seen in lakes distributed across

two biophysical regions in Maine, leading to the conclusion that widespread fish introductions likely have led to regional changes in native aquatic biodiversity and the decline of a unique aquatic ecosystem.

5.3 Management Tools

Many of Maine's lakes are remote and difficult to sample with traditional fish survey methods. As a result, fish surveys have been conducted in less than one quarter of Maine's 6000+ lakes, and many of these have been one-time surveys affording a snapshot of fish community attributes at the time of survey. Current fish presence, however, does not necessarily indicate historical conditions, as not all fish introductions have been documented. Tools to efficiently detect lakes across the landscape (i.e., GIS) that likely are naturally fishless and to assess historical (i.e., paleolimnological assessment) and current (i.e., macroinvertebrate bioindicators) fish absence in these lakes are outlined below. Managers can use these tools to identify lakes for restoration that currently contain fish but that likely were naturally fishless in the past.

5.3.1 Predictive modeling

I developed a method to remotely predict the location of naturally fishless lakes with GIS and to determine the likelihood of historical fish absence with paleolimnogical records in lake sediments (see section 5.2.1; Schilling *et al.*, 2008*a*). Identifying lakes likely to be naturally fishless based on available spatial data layers in a GIS more efficiently and comprehensively identifies potentially fishless lakes than manual map assessment or anecdotal methods. GIS simultaneously assesses many lakes over broad geographic areas, and lakes are assigned a probability of natural fish absence based on

their geomorphic and geographic characteristics. Site surveys can be targeted at lakes with high probability of fish absence, followed by paleolimnological assessment of the sediment record to confirm historical fish absence in lakes with unknown or poorly documented fish stocking histories. This combined approach is valuable in lake rich regions, such as Maine, where widespread and often undocumented fish introductions have occurred, and is a cost effective alternative to large scale and untargeted lake survey efforts.

5.3.2 Indicator surveys

I developed a method to efficiently assess current fish absence by collecting macroinvertebrate taxa (*Graphoderus liberus, Dineutus* spp*., Hesperocorixa* spp*., Callicorixa* spp*., Chaoborus americanus, Notonecta insulata*) that are absent or occur rarely in fish-containing lakes but are widespread in fishless lakes (Schilling *et al.*, 2008*b*). These fishless bioindicators are most successfully collected with nocturnally deployed submerged traps "baited" with a light source. This method for collecting fishless bioindicators is superior to littoral sweeps, because passive collection techniques are more effective for capturing active swimmers that may evade capture in littoral sweeps. Light trap samples also contain no debris or detritus, requiring less processing than littoral sweeps to extract specimens. Confirming fish absence based on bioindicator taxa collection also is more efficient than traditional fish surveys. Although both methods require an overnight sampling protocol, gillnets require a permit, are labor intensive both in equipment and man-power, and cause mortality of fish, if captured. These risks are avoided by submerged light traps.

5.4 Management recommendations

Resource management plans that include stocking naturally fishless lakes must account for the ecological costs to these unique systems in the cost-benefit analysis. My research describes specific changes to macroinvertebrate communities, yet it can be inferred that negative effects of introducing fish into Maine's fishless lakes likely extend beyond those responses examined in this study. Research in western North America shows that introduced fish in historically fishless lakes have direct predatory effects on other aquatic organisms [e.g. zooplankton (Stoddard, 1987; Bradford *et al.*, 1998; Knapp *et al.*, 2001), amphibians (Fisher & Shaffer, 1996; Bradford *et al.*, 1998; Knapp *et al.*, 2001; Pilliod & Peterson, 2001; Denoel *et al.*, 2005; Orizaola & Brana, 2006)], as well as indirect effects on ecosystem processes [e.g. disruption of nutrient cycling and primary productivity (Leavitt *et al.*, 1994; Schindler *et al.*, 2001)] and trophic connections in watersheds [e.g. severing connections between aquatic and terrestrial food webs with cascading effects on riparian plants (Knight *et al.*, 2005), adult amphibians (Finlay & Vredenburg, 2007), birds (P. Epanchin, unpublished), and reptiles (Matthews & Knapp, 2002; Knapp *et al.*, 2007)]. On this basis, fishless lakes warrant a precautionary management approach. Specific management recommendations to protect currently fishless lakes and restore historically fishless lakes, either passively or actively, are outlined below.

5.4.1 Protect currently fishless lakes

Stocking lakes that currently are fishless should be avoided. These lakes should be monitored with periodic fishless bioindicator surveys to verify continued fish absence. Bait bucket stocking and illegal introductions are a constant threat, particularly in easily

accessed lakes near populated areas. Several historically fishless lakes that currently contain fish lack stocking documentation (especially kettle lakes in eastern Maine), indicating illegal fish stocking into these systems. Enhanced enforcement of anti-stocking laws may be required in areas with easily accessed lakes to protect these lakes from illegal fish introductions.

5.4.2 Passively restore historically fishless lakes

Lakes where invertebrate communities have been simplified by stocking should be restored to their natural fishless condition to reestablish their historic landscape distribution and their role in maintaining aquatic biodiversity (see section 5.5.2). A cost-effective passive approach will be successful in lakes that do not sustain naturally reproducing fish populations. If stocking is halted, these lakes will revert to being fishless. This approach will be most effective in headwater lakes where annually stocked brook trout is the only fish species present. Verifying that these populations are not self-sustaining is required to ensure the success of passive restoration. The time required for community recovery may be less in lakes with shorter stocking histories, because communities are less altered in these lakes compared to those in lakes with long stocking histories (Schilling *et al.*, in prep).

5.4.3 Actively restore historically fishless lakes

Restoring lakes with naturally reproducing fish populations will require fish removal. Because many historically fishless kettle lakes are known to support naturalized fish populations, this likely will be the most effective approach for their restoration. Repeated removal efforts and surveys may be required before fish are absent; abundant

habitat structure in these lakes may inhibit fish removal. Recovery efforts in historically fishless lakes in western North America demonstrate that gillnets effectively remove fish, particularly in small lakes (Knapp & Matthews, 1998; Parker *et al.*, 2001). Large, deep lakes may require alternative methods of fish removal [e.g. electrofishing, trap-netting on spawning grounds, disturbing spawning habitat, application of piscicides (Knapp & Matthews, 1998; Parker *et al.*, 2001)].

Although fishless lake recovery has not been documented in Maine, restored fishless lakes in western North America demonstrate the potential for native communities to recover following fish removal (Drake & Naiman, 2000; Donald *et al.*, 2001; Parker *et al.*, 2001; Hoffman *et al.*, 2004; Vredenburg, 2004; Knapp *et al.*, 2005; Knapp *et al.*, 2007). Surveys with submerged light traps can document recovery of the macroinvertebrate community in Maine's fishless lakes. Fish removal accomplished in an experimental framework permits assessment of patterns and timing of fishless lake recovery.

5.5 Conclusions

5.5.1 Resolving conflicting management goals

The Maine Department of Inland Fisheries and Wildlife is faced with potentially conflicting management goals for fishless lakes. As described in their vision statement, MDIFW (2007) strives to: 1) conserve and protect Maine's fisheries and wildlife, and 2) increase opportunities for the use of these resources by all people. To achieve the former, fishless lakes can be protected for their intrinsic ecological value and preserved as a unique resource for native aquatic fauna that evolved in the absence of fish. To achieve the latter, fishless lakes can be managed as potential habitat for native and highly valued

game fish species (e.g. brook trout), with the opportunity to enhance recreational fishing opportunities regionally. These conflicting goals are an obstacle to protecting Maine's fishless lakes and may lead to institutional resistance to changing management policies for these systems.

One way to overcome this resistance is for managers to weigh the costs associated with fishless lake protection against the costs associated with maintaining the state's current stocking program, and to decide how this fits with the agency's mission. Are the ecological costs (i.e., loss of biodiversity, loss of unique habitat) and financial costs (i.e., expense of repeated stocking) of stocking fishless lakes outweighed by the societal and financial benefits of stocking (i.e., enhancement of Maine's fisheries, financial gain from angling licenses sold to those who expect fish in these lakes)?

This research identifies ecological costs of stocking naturally fishless lakes. The financial costs of continued stocking include production of hatchery reared fish and transport to the lakes. Many currently stocked lakes are remote with limited road access and require stocking by airplane. The number of anglers who visit remote lakes likely is small and limited to those able to hike or use ATVs. For anglers seeking wilderness fishing experiences, Maine is a lake-rich state with many remote lakes where fish naturally occur. A moratorium on stocking fishless lakes likely would not disrupt statewide angling expectations and should not result in a decline in fishing license sales. The financial costs associated with protecting Maine's fishless lakes will be both short term (fish removal) and long term (monitoring and enforcement), but also can be minimized (cease stocking). Once the initial costs of fish removal are incurred, there will be minimal costs for monitoring with the submerged light trapping techniques described

above. Funds currently used to stock fishless lakes could be re-allocated to cover these expenses as well as those associated with educating the public about this unique natural system.

5.5.2 Importance of conserving alternative freshwater habitat types

Lentic freshwater habitats in temperate regions exist along a gradient of waterbody permanence and predator presence, ranging from temporary vernal pools lacking large dragonfly and fish predators to permanent lakes where fish are top predators (Wellborn *et al.*, 1996; Stoks & McPeek, 2003). Different habitat types along this gradient are not ecologically redundant; habitat specialization among aquatic organisms is strong (Wellborn *et al.*, 1996). Community structure is determined by both physical factors (e.g. pond drying, winter anoxia) that limit the potential breadth of species distributions and biotic effects (principally predation) mediated by ecological interactions that determine the realized success of species (Wellborn *et al.*, 1996). Ecologists have long recognized this gradient in abiotic and biotic characteristics as a critical axis along which aquatic communities are organized (Brooks & Dodson, 1965; Wiggins *et al.*, 1980; Kenk, 1982).

Critical fitness tradeoffs relating to body size, development, activity, and life history restrict taxa to inhabiting only a subset of the available environmental gradient (Wellborn *et al.*, 1996). Success at one point along the gradient entails having a phenotype that will hinder performance at other points along the gradient (Wellborn *et al.*, 1996). Some taxa persist in only one habitat type, and others use multiple habitats types (Werner & McPeek, 1994; Skelly, 1995; Wellborn *et al.*, 1996; Stoks & McPeek, 2003). For example, *Lestes* and *Enallagma* damselfly assemblages segregate along the

gradient with little overlap of species' use among habitat types, whereas the large dragonfly predator *Anax* is abundant in both semi-permanent ponds and permanent fishless lakes (McPeek, 1990*a*; Stoks & McPeek, 2003). The same ecological mechanisms that limit species distributions may serve as important evolutionary agents of selection for aquatic taxa, driving adaptive evolution and forming a template for lineage diversification (Wellborn *et al.*, 1996; McPeek & Brown, 2000; Stoks & McPeek, 2006). Thus, the availability of different habitat types along the gradient is key to both maintaining and generating aquatic biodiversity across the landscape (Stoks & McPeek, 2003). This perspective does not emerge, however, if each habitat type is examined in isolation and without regard to its location in the landscape.

Viewed in this context, permanent fishless lakes provide a unique habitat niche for organisms that are both unable to withstand periodic drying and unable to coexist with fish. In addition to their value for freshwater biodiversity, fishless lakes play an important functional role in watersheds with respect to transfer of biomass and prey items to riparian zones (Matthews & Knapp, 2002; Finlay & Vredenburg, 2007; Knapp *et al.*, 2007; P. Epanchin, unpublished). This habitat type has been all but eliminated from the gradient of freshwater habitats in many temperate regions worldwide, including Maine (Donald, 1987; Bahls, 1992; Pister, 2001; Denoel *et al.*, 2005; Schilling *et al.*, 2008*a*). Conservation and restoration of fishless lakes is imperative for assuring the persistence of their associated species and communities as well as their functional roles in watersheds. Recognizing the ecological value of fishless lakes and their potential for recovery has spurred state and federal agencies in western North America to halt stocking and begin restoration (Milliron, 1999; Yosemite National Park, 2006; Bunn *et al.*, 2007). There

have been no similar attempts to mitigate the effects of stocking historically fishless lakes in northeastern North America, and the Maine Department of Inland Fisheries and Wildlife has the opportunity to take the lead in this effort.

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APPENDIX : ADDITIONAL DATA

Table A.1. Fishless and fish-containing study lakes in Chapter 2, including physical lake attributes and fish species composition.

Midas	*Lake name*	*Region*	*Fish species composition*	*Elevation (m)*	*Area (ha)*	*pH*	*Maximum depth (m)*
1190	Black Brook #4	east	fishless	58	1.52	4.44	12.8
4474	Duck Pd	east	fishless	80	3.74	4.5	4.9
219	Kerosene Pd	east	fishless	71	2.63	4.49	5.2
4420	Mud Pd 4420	east	fishless	103	1.57	4.88	15.8
1222	Oak Pd	east	fishless	74	0.77	5.29	0.6
8385	Unnamed8385	east	fishless	140	7.65	4.15	6.7
9629	Unnamed9629	east	fishless	70	1.40	4.66	5.2
9633	Unnamed9633	east	fishless	78	1.39	5.18	2.4
8064	Apple Pd	west	fishless	254	1.77	6.77	9.8
906	Cloud Pd	west	fishless	729	6.36	5.07	8.5
8603	Cranberry Pd	west	fishless	754	2.80	6.27	4.6
3592	Jackson Pd	west	fishless	893	0.88	7.24	1.5
554	Loon Pd 554	west	fishless	432	1.75	6.04	2.4
890	Midday Pd	west	fishless	405	4.91	6.23	11.9
3284	North Pd	west	fishless	478	2.99	5.55	0.9
8340	Unnamed8340	west	fishless	541	1.83	6.98	7.3
4590	Crocker Pd	east	golden shiner, fathead minnow	73	2.23	6.77	2.7
4778	Crystal Pd	east	brook trout, golden shiner, fathead minnow	112	8.45	5.78	13.4
453	Loon Pd 453	east	brook trout, golden shiner, fathead minnow	110	4.38	6.09	11.0
4587	Pickerel Pd	east	brook trout, golden shiner, brown bullhead, chain pickerel	79	4.72	5.92	7.3
3	Pineo Pd	east	brook trout, golden shiner, threespine stickleback	62	2.85	6.04	15.8
1158	Salmon Pd 1158	east	brook trout, golden shiner, landlocked Atlantic salmon	67	3.91	6.45	6.7
4422	Salmon Pd 4422	east	brook trout, golden shiner, banded killifish, pumpkinseed	88	2.59	5.01	10.7
4374	Simmons Pd	east	brook trout, golden shiner, brown bullhead, smallmouth bass, white sucker	55	5.21	5.5	8.2
7537	Unnamed7537	east	pumkinseed	79	2.14	5.78	1.2
8417	Unnamed8417	east	golden shiner	75	1.02	6.34	1.2
340	Bell Pd	west	brook trout	388	7.47	5.41	8.5
464	Greenwood Pd	west	brook trout	677	7.36	6.01	12.8
556	Hedgehog Pd	west	finescale dace	406	1.92	6.75	0.9
704	Jackson Pd #2	west	brook trout, northern redbelly dace, finescale dace	376	3.57	5.17	2.1
786	Notch Pd	west	brook trout	567	2.19	6.07	7.3
3542	Rock Pd	west	brook trout	831	2.00	5.84	4.6
5060	Snow Mountain Pd	west	brook trout	854	4.03	6.38	2.1
3286	York Pd	west	brook trout, golden shiner, black nose dace, creek chub	479	2.81	6.38	2.1

Table A.2. Taxonomic keys used to identify macroinvertebrates.

Taxa identified	*Key(s) used*
All taxa to genus	Merritt & Cummins, 1996
Coleoptera to species	Downie & Arnett, 1996
Dytiscidae to species	Larson *et al.*, 2000
	Boobar *et al.* 1998
Odonata to species	Westfall & May, 1996
	Needham *et al.*, 2000
Notonectidae to species	Hungerford, 1933
	Truxel, 1953
Chaoborus to species	Saether, 1970
	Borkent, 1979
Corixidae to genus	Hungerford, 1948

Table A.3. Mean abundance [$\log_{10}(x+1)$ transformed] (standard error) and percent occurrence of common Hemiptera and Coleoptera [(A) & (B)] and Odonata, Diptera and Ephemeroptera [(C) &(D)] collected in light traps and littoral sweeps in fishless and fish-containing lakes in Chapter 2. Test statistics and p-values are given testing differences between lake types in abundance (Student's *t*-tests) and percent occurrence [Pearson Chi-square (when expected values were >5) and Fisher's Exact Tests (when expected values were ≤ 5)]. Highlighted cells indicate significant associations with fishless lakes (light grey) and fish-containing lakes (dark grey). No test statistic is generated with Fisher's Exact Tests; df = 1 for all 2x2 contingency tables.

(A)

		Light trap collections								
		Abundance					*Percent occurrence*			
Order		Fishless lakes (SE)	Fish-containing lakes (SE)	t	df	p	Fishless lakes	Fish-containing lakes	Pearson $\chi 2$	p
Hemiptera	Notonectidae	1.74 (0.25)	0.30 (0.09)	-5.42	19	0.000	93.75	50.00	na	0.008
	Buenoa	1.03 (0.26)	0.20 (0.08)	-3.04	18	0.007	68.75	33.33	4.25	0.039
	Buenoa macrotibialis	0.61 (0.23)	0.11 (0.06)	-2.10	17	0.051	37.50	22.22	na	0.457
	Notonecta	1.19 (0.27)	0.10 (0.06)	-4.01	16	0.001	75.00	16.67	11.69	0.001
	Notonecta insulata	0.52 (0.15)	0.00 (0.00)	-3.56	15	0.003	56.25	0.00	na	0.000
	Notonecta undulata	**not commonly captured**								
	Corixidae	1.95 (0.15)	1.22 (0.21)	-2.73	32	0.010	100.00	94.44	na	1.000
	Callicorixa	0.35 (0.11)	0.00 (0.00)	-3.25	15	0.005	50.00	0.00	na	0.001
	Cenocorixa	0.24 (0.09)	0.70 (0.18)	2.35	25	0.027	37.50	83.33	7.54	0.006
	Graptocorixa	0.04 (0.03)	0.03 (0.02)	-0.12	32	0.904	12.50	11.11	na	1.000
	Hesperocorixa	0.68 (0.14)	0.05 (0.04)	-4.39	17	0.000	75.00	11.11	14.28	0.000
	Neocorixa	0.31 (0.15)	0.06 (0.03)	-1.64	17	0.120	31.25	16.67	na	0.429
	Palmacorixa	0.03 (0.03)	0.36 (0.21)	1.58	18	0.132	6.25	27.78	na	0.180
	Sigara	0.87 (0.16)	0.30 (0.11)	-2.97	32	0.006	75.00	38.89	4.48	0.034
	Belostomatidae	0.30 (0.09)	0.19 (0.08)	-0.93	32	0.358	50.00	27.78	1.77	0.183
	Lethocerus	0.24 (0.09)	0.12 (0.07)	-1.11	32	0.277	37.50	16.67	na	0.250
	Nepidae	0.09 (0.06)	0.06 (0.03)	0.05	25	0.958	12.50	16.67	na	1.000
	Ranatra	0.06 (0.06)	0.06 (0.03)	0.05	25	0.958	6.25	16.67	na	0.604
	Gerridae	0.00 (0.00)	0.09 (0.04)	2.13	17	0.048	0.00	22.22	na	0.105
Coleoptera	Gyrinidae	0.95 (0.16)	0.16 (0.08)	-4.33	22	0.000	87.50	27.78	12.26	0.000
	Dineutus	0.69 (0.17)	0.09 (0.07)	-3.33	21	0.003	68.75	11.11	11.92	0.001
	Oreodytes	**not commonly captured**								
	Gyrinus	0.50 (0.13)	0.07 (0.04)	-3.07	18	0.007	68.75	16.67	9.49	0.002
	Dytiscidae	1.60 (0.19)	0.19 (0.60)	-2.19	17	0.042	93.75	44.44	9.41	0.002
	Dytiscus	0.34 (0.13)	0.05 (0.04)	-2.19	17	0.042	37.50	11.11	na	0.110
	Graphoderus	1.51 (0.20)	0.09 (0.05)	-6.90	17	0.000	93.75	16.67	20.20	0.000
	Graphoderus liberus	1.09 (0.15)	0.00 (0.00)	-7.08	15	0.000	93.75	0.00	30.20	0.000
	Ilybius	0.16 (0.06)	0.00 (0.00)	2.13	15	0.019	37.50	0.00	na	0.006
	Ilybius discedens	0.16 (0.06)	0.00 (0.00)	-2.62	15	0.019	37.50	0.00	na	0.006
	Laccophilus	0.07 (0.04)	0.03 (0.02)	-0.78	25	0.446	18.75	11.11	na	0.648
	Laccophilus maculosus	0.07 (0.04)	0.03 (0.02)	-0.59	27	0.561	18.75	11.11	na	0.648
	Matus	0.14 (0.06)	0.03 (0.02)	-1.62	20	0.121	31.25	11.11	na	0.214
	Matus ovatus	0.12 (0.06)	0.03 (0.02)	-1.33	20	0.198	25.00	11.11	na	0.387
	Thermonectes	0.15 (0.07)	0.00 (0.00)	2.04	32	0.036	25.00	0.00	na	0.039
	Haliplidae	0.32 (0.06)	0.42 (0.09)	0.93	29	0.359	75.00	61.11	0.75	0.388
	Haliplus	0.27 (0.06)	0.39 (0.09)	1.10	28	0.279	68.75	55.56	0.62	0.429
	Haliplus blanchardi	0.19 (0.05)	0.33 (0.08)	1.50	27	0.146	56.25	55.56	0.00	0.968
	Haliplus immaculicollis	0.03 (0.03)	0.07 (0.04)	0.74	32	0.463	6.25	16.67	na	0.347
	Haliplus longulus	0.05 (0.03)	0.03 (0.02)	-0.38	32	0.709	12.50	11.11	na	1.000
	Haliplus leopardus	**not commonly captured**								
	Peltodytes	0.08 (0.04)	0.08 (0.04)	0.14	32	0.889	18.75	22.22	na	1.000
	Peltodytes pedunculatus	0.04 (0.04)	0.08 (0.04)	0.75	32	0.458	6.25	22.22	na	0.340
	Hydrophilidae	0.08 (0.03)	0.05 (0.03)	-0.6	32	0.56	25.00	16.67	na	0.681
	Tropisternus	0.06 (0.03)	0.03 (0.02)	-0.61	32	0.544	18.75	11.11	na	0.648
	Tropisternus mixtus	0.06 (0.03)	0.03 (0.02)	-0.61	29	0.550	18.75	11.11	na	0.648

(B)

Littoral sweep collections

Order		*Abundance*					*Percent occurrence*		
		Fishless lakes (SE)	Fish-containing lakes (SE)	t	df	p	Fishless lakes	Fish-containing lakes	p
Hemiptera	Notonectidae	0.88 (0.27)	0.18 (0.10)	-2.44	13	0.030	54.55	27.27	0.387
	Buenoa	0.36 (0.18)	0.10 (0.07)	-1.42	13	0.180	36.36	18.18	0.636
	Buenoa macrotibialis	not commonly captured							
	Notonecta	0.82 (0.25)	0.15 (0.08)	-2.54	12	0.026	54.55	27.27	0.387
	Notonecta insulata	not commonly captured							
	Notonecta undulata	0.35 (0.18)	0.06 (0.06)	-1.53	13	0.151	80.00	20.00	0.311
	Corixidae	not identified							
	Callicorixa								
	Cenocorixa								
	Graptocorixa								
	Hesperocorixa								
	Neocorixa								
	Palmacorixa								
	Sigara								
	Belostomatidae								
	Lethocerus								
	Nepidae								
	Ranatra								
	Gerridae								
Coleoptera	Gyrinidae	0.21 (0.10)	0.03 (0.03)	-1.73	12	0.111	36.36	9.09	0.311
	Dineutus	not commonly captured							
	Oreodytes	0.08 (0.06)	0.05 (0.05)	-0.34	20	0.737	18.18	9.09	1.000
	Gyrinus	0.19 (0.10)	0.03 (0.03)	-1.62	12	0.132	36.36	9.09	0.311
	Dytiscidae	0.49 (0.21)	0.15 (0.09)	-1.48	13	0.164	36.36	27.27	1.000
	Dytiscus	0.06 (0.06)	0.05 (0.04)	-0.12	20	0.906	9.09	18.18	1.000
	Graphoderus	0.47 (0.20)	0.00 (0.00)	-2.29	10	0.045	36.36	0.00	0.090
	Graphoderus liberus	0.21 (0.10)	0.00 (0.00)	-2.21	10	0.052	100.00	0.00	0.090
	Ilybius	not commonly captured							
	Ilybius discedens								
	Laccophilus								
	Laccophilus maculosus								
	Matus								
	Matus ovatus								
	Thermonectes								
	Haliplidae	0.15 (0.09)	0.10 (0.07)	-0.459	20	0.651	27.27	18.18	1.000
	Haliplus	0.13 (0.07)	0.10 (0.07)	-0.29	29	0.776	27.27	18.18	1.000
	Haliplus blanchardi	not commonly captured							
	Haliplus immaculicollis								
	Haliplus longulus								
	Haliplus leopardus	0.07 (0.05)	0.03 (0.03)	-0.774	16	0.451	66.67	33.33	1.000
	Peltodytes	not commonly captured							
	Peltodytes pedunculatus								
	Hydrophilidae								
	Tropisternus								
	Tropisternus mixtus								

(C)

Order		Light trap collections: Abundance: Fishless lakes (SE)	Abundance: Fish-containing lakes (SE)	Abundance: t	Abundance: df	Abundance: p	Percent occurrence: Fishless lakes	Percent occurrence: Fish-containing lakes	Percent occurrence: Pearson $\chi 2$	Percent occurrence: p
Odonata	Aeshnidae	0.44 (0.12)	0.11 (0.05)	-2.55	21	0.019	62.50	22.22	5.67	0.017
	Aeshna	0.43 (0.12)	0.09 (0.04)	-2.71	19	0.014	62.50	22.22	5.67	0.017
	Aeshna eremita	0.26 (0.09)	0.04 (0.03)	-2.18	18	0.043	43.75	11.11	na	0.052
	Aeshna interrupta	0.18 (0.10)	0.03 (0.02)	-1.40	17	0.180	25.00	11.11	na	0.387
	Corduliidae	not commonly captured								
	Cordulia									
	Cordulia shurtleffi									
	Libellulidae	0.39 (0.15)	0.12 (0.08)	-1.680	23	0.106	43.750	16.68	na	0.134
	Ladona	not commonly captured								
	Ladona julia									
	Leucorrhinia	0.38 (0.14)	0.12 (0.08)	-1.61	23	0.122	43.75	16.67	na	0.134
	Leucorrhinia glacialis	not commonly captured								
	Leucorrhinia hudsonica	0.18 (0.09)	0.07 (0.07)	-0.91	21	0.369	25.00	5.56	na	0.164
	Coenagrionidae	0.83 (0.20)	0.33 (0.10)	-2.22	23	0.037	68.75	44.44	2.03	0.154
	Enallagma	0.22 (0.07)	0.20 (0.10)	-0.17	32	0.867	50.00	27.78	1.77	0.183
	Lestidae	0.21 (0.10)	0.19 (0.08)	-0.19	32	0.852	25.00	27.78	na	1.000
	Lestes	0.21 (0.10)	0.19 (0.08)	-0.14	32	0.887	25.00	27.78	na	1.000
	Lestes rectangularis	0.10 (0.07)	0.06 (0.06)	-0.45	32	0.653	18.75	5.56	na	0.323
	Lestes unguiculatus	0.12 (0.06)	0.05 (0.04)	-1.11	32	0.277	25.00	11.11	na	0.387
Diptera	*Chaoborus*	1.02 (0.13)	0.91 (0.22)	-0.41	27	0.688	87.50	66.67	na	0.233
	Chaoborus albatus	0.29 (0.15)	0.14 (0.12)	-0.82	32	0.420	25.00	11.11	na	0.387
	Chaoborus americanus	0.54 (0.13)	0.00 (0.00)	-4.23	15	0.000	62.50	0.00	15.94	0.000
	Chaoborus punctipennis	0.21 (0.10)	0.82 (0.23)	2.45	24	0.022	25.00	55.56	3.27	0.071
Ephemeroptera	Ameletidae	0.04 (0.04)	0.16 (0.09)	1.20	25	0.241	6.25	22.22	na	0.340
	Ameletus	0.04 (0.04)	0.16 (0.09)	1.20	25	0.241	6.25	22.22	na	0.340
	Siphlonuridae	0.16 (0.12)	0.11 (0.06)	-0.37	22	0.715	12.50	22.22	na	0.660
	Siphlonurus	0.11 (0.11)	0.06 (0.03)	-0.45	18	0.662	6.25	16.67	na	0.604

(D)

Order		Littoral sweep collections: Abundance: Fishless lakes (SE)	Abundance: Fish-containing lakes (SE)	Abundance: t	Abundance: df	Abundance: p	Percent occurrence: Fishless lakes	Percent occurrence: Fish-containing lakes	Percent occurrence: p
Odonata	Aeshnidae	0.86 (0.14	0.46 (0.13)	-2.16	20	0.043	90.91	63.64	0.311
	Aeshna	0.68 (0.14)	0.30 (0.09)	-2.21	20	0.039	90.91	54.55	0.149
	Aeshna eremita	0.31 (0.08)	0.07 (0.05)	-2.53	20	0.020	77.78	22.22	0.080
	Aeshna interrupta	not commonly captured							
	Corduliidae	0.43 (0.10)	0.40 (0.125)	-0.189	29	0.852	72.73	63.64	1.000
	Cordulia	0.36 (0.11)	0.25 (0.09)	-0.78	20	0.447	63.64	54.55	1.000
	Cordulia shurtleffi	0.36 (0.11)	0.25 (0.09)	-0.78	20	0.447	53.85	46.15	1.000
	Libellulidae	1.25 (0.22)	0.78 (0.16)	-1.71	20	0.103	100.00	81.82	0.476
	Ladona	0.10 (0.05)	0.21 (0.09)	1.07	20	0.259	27.27	45.46	0.659
	Ladona julia	0.10 (0.05)	0.21 (0.09)	1.07	20	0.296	37.50	62.50	0.659
	Leucorrhinia	1.20 (0.22)	0.41 (0.15)	-2.96	20	0.008	100.00	54.55	0.035
	Leucorrhinia glacialis	1.20 (0.24)	0.27 (0.10)	-3.45	14	0.004	66.67	33.33	0.063
	Leucorrhinia hudsonica	not commonly captured							
	Coenagrionidae								
	Enallagma								
	Lestidae								
	Lestes								
	Lestes rectangularis								
	Lestes unguiculatus								
Diptera	*Chaoborus*	not identified							
	Chaoborus albatus								
	Chaoborus americanus								
	Chaoborus punctipennis								
Ephemeroptera	Ameletidae								
	Ameletus								
	Siphlonuridae								
	Siphlonurus								

Table A.4. Fishless and stocked study lakes in Chapter 3, including physical lake attributes and fish species composition. Evidence of fish absence was based on fish surveys conducted at time of study ("Current"), paleolimnological assessment of lake sediments (Schilling *et al.* 2008*a*; DeGoosh 2007), or historic fish survey records for lakes documented by MDIFW as fishless prior to initial introduction of brook trout (year of introduction is indicated).

Midas	*Lake name*	*Lake type*	*Elevation (m)*	*Area (ha)*	*pH*	*Maximum depth (m)*	*Fish species composition (year of introduction)*	*Evidence of fish absence*
8064	Apple Pd	headwater	254	1.77	6.77	9.75	fishless	Current
906	Cloud Pd	headwater	729	6.36	5.07	8.53	fishless	Current
8603	Cranberry Pd	headwater	754	2.80	6.27	4.57	fishless	Current
3592	Jackson Pd	headwater	893	0.88	7.24	1.52	fishless	Current
554	Loon Pd 554	headwater	432	1.75	6.04	2.44	fishless	Current
890	Midday Pd	headwater	405	4.91	6.23	11.89	fishless	Current
3284	North Pd	headwater	478	2.99	5.55	0.91	fishless	Current
8340	Unnamed8340	headwater	541	1.83	6.98	7.32	fishless	Current
3288	Speck Pd	headwater	1039	4.07	5.32	10.97	brook trout* (1962)	Historic fish survey records
3554	Ledge Pd	headwater	893	1.65	6.12	7.32	brook trout* (1964)	Historic fish survey records
3512	Tumbledown Pd	headwater	817	3.24	5.71	6.71	brook trout* (1966), golden shiner†	Historic fish survey records
2636	Beaver Pd	headwater	488	5.29	6.85	3.66	brook trout* (1999)	Historic fish survey records
384	Lily Pd	headwater	343	4.71	6.54	11.58	brook trout* (1999)	Historic fish survey records
786	Notch Pd	headwater	567	2.19	6.07	7.32	brook trout	Paleolimnological assessment
5060	Snow Mountain Pd	headwater	854	4.03	6.38	2.13	brook trout	Paleolimnological assessment
8601	The Horns Pd	headwater	954	1.33	5.91	7.01	brook trout*	Paleolimnological assessment
1190	Black Brook #4	kettle	58	1.52	4.44	12.80	fishless	Current
9629	Unnamed9629	kettle	70	1.40	4.66	5.18	fishless	Current
219	Kerosene Pd	kettle	71	2.63	4.49	5.18	fishless	Current
1222	Oak Pd	kettle	74	0.77	5.29	0.61	fishless	Current
9633	Unnamed9633	kettle	78	1.39	5.18	2.44	fishless	Current
4474	Duck Pd	kettle	80	3.74	4.5	4.88	fishless	Current
4420	Mud Pd 4420	kettle	103	1.57	4.88	15.85	fishless	Current
8385	Unnamed8385	kettle	140	7.65	4.15	6.71	fishless	Current
4587	Pickerel Pd	kettle	79	4.72	5.92	7.32	brook trout*, brown bullhead, golden shiner, chain pickerel	Paleolimnological assessment
1158	Salmon Pd 1158	kettle	67	3.91	6.45	6.71	brook trout*, golden shiner, landlocked salmon	Paleolimnological assessment
4374	Simmons Pd	kettle	55	5.21	5.50	8.23	brook trout, brown bullhead, golden shiner†, small mouth bass, white sucker	Paleolimnological assessment
4590	Crocker Pd	kettle	73	2.23	6.77	2.74	fathead minnow, golden shiner	Paleolimnological assessment
8417	Unnamed8417	kettle	75	1.02	6.34	1.22	golden shiner	Paleolimnological assessment
7537	Unnamed7537	kettle	79	2.14	5.78	1.22	pumkinseed	Paleolimnological assessment

BIOGRAPHY OF THE AUTHOR

Emily Gaenzle Schilling was born in Rochester, NY in August, 1975. She attended Brighton High School in Rochester, NY and pursued her undergraduate studies at Colgate University in Hamilton, NY, where graduated magna cum laude and Phi Beta Kappa in May 1997, with a B.A. in Biology and French. Following graduation she received a Fulbright Teaching Assistantship and taught English in secondary schools in Besançon, France during 1997 – 1998. She returned to the USA to work at the American Museum of Natural History as the International Field Programs Coordinator for the Center for Biodiversity and Conservation from 1998 – 1999. She taught Biology at the Grace Church School in New York from 1999 – 2000. She began her graduate studies at the University of Maine in 2000 and received her M.S. in Ecology and Environmental Science in the fall of 2002. Her Master's work focused on understanding Her relationships between stream geomorphology and fish community structure.

Emily was awarded a National Science Foundation Graduate Research Fellowship and began her Ph.D. work in the summer of 2002, advised by Dr. Cynthia Loftin. Her dissertation research focuses on the physical and biological attributes of naturally fishless lakes in Maine. Emily is a candidate for the Doctor of Philosophy degree in Ecology and Environmental Science from The University of Maine in August, 2008.